

# Iron Overload in Beta Thalassemia Major and Intermedia Patients in West Bengal

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## ABSTRACT

**Background:** In  $\beta$ -thalassemia, the erythropoietic process is markedly altered, and the lack or reduced synthesis of  $\beta$ -globin chains induces an excess of free  $\alpha$ -globin chains within the erythroid cells. Aggregation, denaturation, and degradation of these chains lead to the formation of insoluble precipitates causing damage to the red blood cell membrane. One of the major consequences in this genetic disorder is iron overload due to ineffective erythropoiesis and premature hemolysis in the plasma and in major organs such as heart, liver, and endocrine glands. Iron homeostasis involves the regulation of cells that export iron into plasma and cells that utilize or store iron. The cellular iron balance in humans is primarily mediated by the hepcidin–ferroportin axis. Ferroportin is the sole cellular iron export protein, and its expression is regulated transcriptionally, post-transcriptionally and post-translationally. Hepcidin, a hormone produced by liver cells, post-translationally regulates ferroportin expression on iron exporting cells by binding with ferroportin and promoting its internalization by endocytosis and subsequent degradation by lysosomes. Dysregulation of iron homeostasis leading to iron deposition in vital organs is the main cause of death in beta-thalassemia patients. Beta-thalassemia patients show marked hepcidin suppression, ineffective erythropoiesis, anemia and iron overload.

**Objective:** The aim of the present study is to assess the serum ferritin levels in multi-transfused Thalassaemia major and Thalassaemia intermedia patients in West Bengal. The study was also done to estimate the present situation of awareness of iron overload in them.

**Methods:** Hundred and eleven blood samples from clinically diagnosed thalassaemia major and intermedia patients were collected from RGKAR Medical College and Hospital in West Bengal for their serum ferritin estimation. Serum ferritin measurement was performed using an indirect enzyme linked immune sorbent based serum ferritin assay kit. Data were analyzed to determine the association between variables. The association between age, sex, and serum ferritin level were established.

**Results:** 72.6% of the beta thalassaemia major patients showed very high ferritin levels. The mean serum ferritin level was found to be 2458 ng/ml. 57.6% (64) patients had serum ferritin between 1000 to 2500 ng/ml, while 42.3% (47) patients had values above 2500 ng/ml. These levels reflect inadequate chelation and vulnerability to develop iron overload related complications.

**Conclusion:** There is an urgent need to rationalize the chelation therapy and to create awareness about the consequences of iron overload in the patients of West Bengal. The study showed high levels of serum ferritin beta thalassaemia major patients which give an overall bleak view

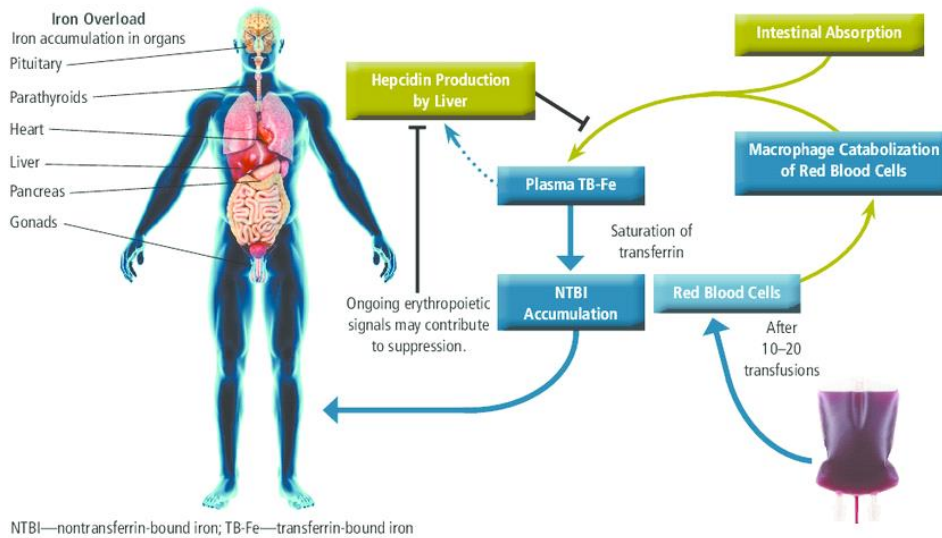
**Keywords:** Serum Ferritin Level; Awareness; Iron chelators

**Introduction:**

Thalassemia is an inherited blood disorder in which the body makes an abnormal form of hemoglobin. Hemoglobin is the protein molecule in red blood cells that carries oxygen. The disorder results in excessive destruction of red blood cells, which leads to anemia. Anemia is a condition in which your body doesn't have enough normal, healthy red blood cells. Thalassemia is inherited, meaning that at least one of your parents must be a carrier of the disorder. It's caused by either a genetic mutation or a deletion of certain key gene fragments. Thalassemia minor is a less serious form of the disorder. There are two main forms of thalassemia that are more serious. In alpha thalassemia, at least one of the alpha globin genes has a mutation or abnormality. In beta thalassemia, the beta globin genes are affected.

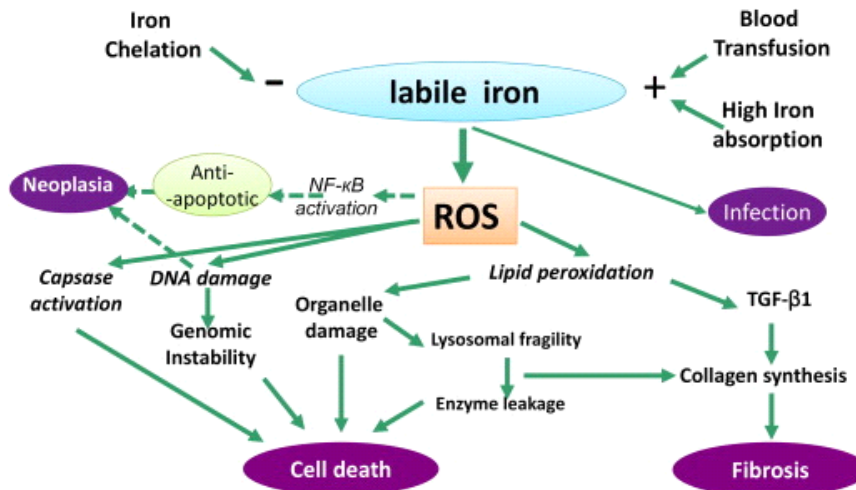
It is caused either by a genetic disorder or by the deletion of certain main segments of the gene. In a cluster of the beta-globin gene on chromosome 11 and the 16 chromosomes on alpha-globin gene cluster molecular defects result in inaccurate production of hemoglobin. Thalassemia conditions with multiple clinical symptoms, phenotypes and therapeutic options are focused on a continuum of severity. TDT (transfusion-dependent thalassemia) and NTDT (non-transfusion dependent thalassemia) are two types of thalassemia. In both transfusion-dependent thalassemia and non-transfusion dependent thalassemia, iron overload is associated with high morbidity. Iron overload is caused by an excess accumulation of intestinal iron confirmed by inadequate erythropoiesis. Excess iron deposition, which begins in the first year of routine blood transfusion, causes harm to many vital organs like heart, liver. For years, arrhythmias have been well documented in the medical arena as a cardiovascular consequence of iron overload (IO). They are thought to be linked to the accumulation of iron in the myocardium. Cardiomyopathy, often caused by iron overload, is a frequent and preventable form of heart failure. With atrial and ventricular tachyarrhythmias, iron accumulation in the heart tissue promotes non-homogeneous electrical conduction and repolarization. The entire cardiac conduction system, particularly the atrioventricular node, may be affected by iron deposition.

Hemochromatosis, also known as iron overload, is characterized by improper iron accumulation in the functional parts of an organ, which results in organ damage and failure. Human bodies store iron, mainly in the form of ferritin. Limited content of ferritin is secreted into bloodstreams. The blood ferritin concentration is checked, in the absence of inflammation, this blood ferritin is linked positively corrected, by total body iron stores. Standard concentrations of ferritin differ by sex and age. Ferritin concentration tends to increase at the age of around one year and even grow in adulthood. Males, however, have a higher level of concentration values than females.



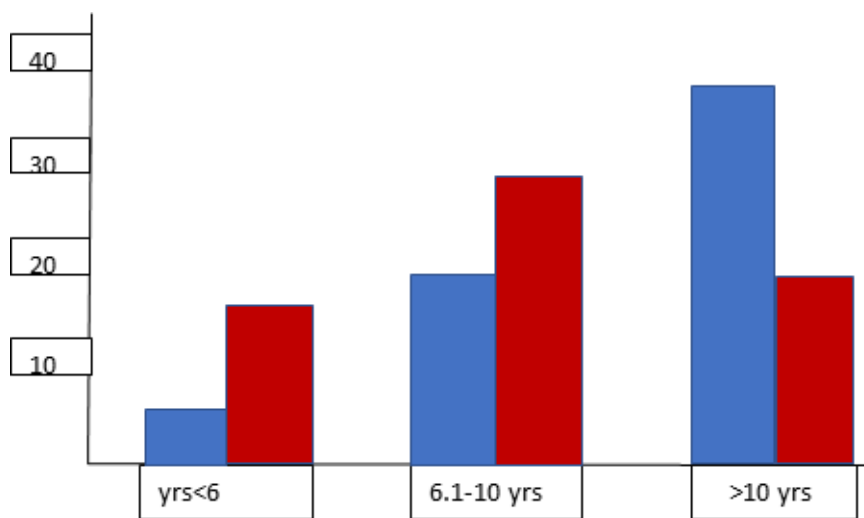
### Pathophysiology of iron overload in beta-thalassemia:

The excess iron in beta-thalassemia patients saturates the ability of the transferrin iron transport system, leading to non-transferrin bound iron (NTBI) and labile plasma iron (LPI) starting to circulate in plasma and subsequently becoming deposited inside susceptible cells. Rather than using the transferrin receptor, NTBI enters cells by other cellular channels including L-type voltage-dependent  $Ca^{2+}$  channel (LVDC), a promiscuous divalent cation transporter and Zip14, a member of the SLC39A zinc transporter family. Long-term uptake and accumulation of NTBI and LPI, its redox active component, leads to increased levels of storage iron and labile cellular iron. Tissues susceptible to iron accumulation by this mechanism include the liver, endocrine system and myocardium. When the magnitude of the cellular labile iron pool exceeds the capacity of the cell to synthesize new ferritin molecules, a critical concentration is reached that can generate reactive oxygen species (ROS). ROS produced by the metabolism of NTBI plays a central role in inducing cellular dysfunction, apoptosis, and necrosis. A variety of ROS, most notably hydroxyl radicals, increase lipid peroxidation and organelle damage, leading to cell death and fibrogenesis mediated by transforming growth factor beta1 (TGF-beta1). An underappreciated effect of iron overload is increased infection risk that is a high cause of mortality in beta-thalassemia patients. Oxidative stress is a major inducer of autophagy, which is important in the removal of oxidized proteins and damaged mitochondria. The increased activation of autophagy has been reported in beta-thalassemia/Hb E erythroblasts as compared to normal control erythroblasts, suggesting that high levels of autophagy in beta-thalassemia/Hb E erythroblasts might be induced by ROS that contribute to the increased levels of apoptosis that lead to ineffective erythropoiesis in beta-thalassemia/Hb E erythroblasts.



**Sample and Center of Study:** A total of 111 blood samples are taken from Beta thalassemia major and beta thalassemia intermedia in this study. The study was conducted at RgKar Medical college and hospital, Kolkata, West Bengal, India.

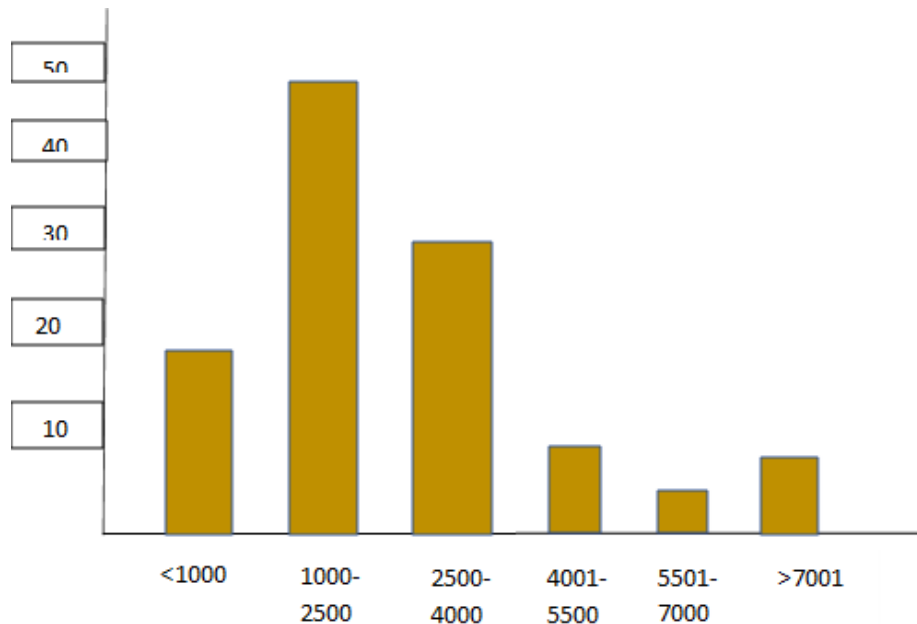
**Sample collection:** The known cases of  $\beta$ -thalassaemia major and thalassaemia intermedia that had been transfused irrespective of their age and gender were randomly selected. Subjects were classified into three age groups: <6 years, in between 6.1-10 years and >10 years. In the given graph below, the red shows the number of females and blue shows number of males in different age categories.



**Clinical Data:** The clinical details of patients were recorded in a proforma, considering the age, gender, frequency of transfusions and estimation of serum ferritin levels.

**Serum Ferritin Estimation:** About 3 ml of the patient's blood sample was collected by a clean venepuncture. The blood was allowed to clot. Serum was separated and stored at  $-20^{\circ}\text{C}$ . Ferritin levels were performed by using indirect enzyme linked immunosorbent assay (ELISA) kit along with normal and abnormal controls. Anti-human-ferritin antibodies were bound to microwells.

**RESULTS:** In this study, a total of 111 cases consisting of 53.1% female and 46.8% male, with the ration of female to male is 1.13:1. The mean age of males were 14.3 years and the mean age of females were 8.2 years. The age at the time of serum ferritin estimation ranged between 3 years to 25 years. The graph displays the range of serum ferritin levels observed in patients. The mean serum ferritin level was 2456 ng/ml. Only 17 patients (15.3%) had serum ferritin level less than 1000 ng/ml. 47 patients (42.3) had serum ferritin level between 1000-2500 ng/ml, while 42.3 patients (47) had values more than 2500 ng/ml .



**DISCUSSION:**

$\beta$ -thalassemia is a significant health problem in various areas of the world due to its frequency and severity. The standard treatment of  $\beta$ -thalassemia is currently based on transfusion therapy, iron chelation, and, in rare cases, splenectomy. This has led to an increased survival and amelioration of the quality of life, although many patients continue to be affected by cardiac disease and other clinical complications, e.g., developed endocrine failure and delayed pubertal maturation. The only approach that may lead to a definitive cure for  $\beta$ -thalassemia is represented by allogenic hemopoietic stem cell transplantation, but the need to control transplant-related complications and the requirement for matched donors make this option not available to most patients. Thus, the main therapeutic option for most patients remains to be supportive care in the form of blood transfusion combined with chelation therapy. The function of iron chelators is that to remove excess iron from the plasma and the cells by binding the labile and chelatable iron, thus facilitating its excretion through the urine and feces. Deferoxamine was the first iron chelator to be used clinically and is given by a slow, continuous, subcutaneous, overnight infusion through a portable pump. Its side effects are minimal, but its mode of administration results in low compliance. Deferasirox presents several side effects. Neutropenia is the main potential complication of deferiprone, the first effective oral iron chelator in removing excess iron from the organs and from the heart. The use of a combination of chelators leads to an improvement in the efficacy of chelation therapy: deferiprone may mobilize iron from tissues into the circulation, while deferoxamine binds and facilitates its excretion in the urine (the “shuttle mechanism”). An additional potential approach to reduce iron overload is the downregulation of transferrin receptor 1 (TfR1) by

administration of exogenous iron-free (apo) transferrin. In addition to free iron, some iron-containing compounds, due to hemolysis, are elevated in the plasma of  $\beta$ -thalassemia patients. They are free heme and hemoglobin and are of considerable toxicity. These compounds are neutralized by their scavengers: hemopexin for free heme and haptoglobin for free hemoglobin. These proteins are reduced in  $\beta$ -thalassemia patients, leaving free, un-neutralized heme, and hemoglobin. The administration of hemopexin and haptoglobin may be suggested to reduce iron toxicity.

### LIMITATIONS

The height and weight (and consequently the body mass index) of the patients was not obtained. The dose and frequency of Deferroxamine infusions or any other iron chelator therapy is not investigated and is also a limitation

### CONCLUSION

The estimation of serum iron can be done using ELISA based techniques. There is a strong need to create awareness among patients about the consequences of iron overload in their body. The high level of serum ferritin of beta thalassaemia major patients noted in this study supports the rationale for regular follow-up of transfusion dependent thalassaemic patients with respect to iron overload to ensure proper management of iron overload associated complications. Proper chelation of iron overload could improve the quality of life of these patients. The problems of poverty, low education level and inadequate provision of health care are the main stumbling blocks in effective treatment of iron overload in thalassaemic patients which is the main cause of morbidity and mortality in thalassaemia major.

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