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Review

Pancreatic changes affecting glucose homeostasis in transfusion dependent β-thalassemia (TDT): a short review

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Abstract. *Background*: The natural history of the glycometabolic state in transfusion-dependent β-thalassemia (TDT) patients is characterized by a deterioration of glucose tolerance over time. *Aims*: This review depicts our current knowledges on the complex and multifacet pathophysiologic mechanisms implicated in the development of alteration of glucose homeostasis in patients with TDT. *Search strategy:* A systematic search was done on December 2020 including Web of Science (ISI), Scopus, PubMed, Embase, and Scholar for papers published in the last 20 years. Moreover, we checked the reference lists of the relevant articles and previously performed reviews for additional pertinent studies. The personal experience on the care of patients with thalassemias is also reported. *Conclusion:* A regular packed red blood cells (PRBCs) transfusion program, optimization of chelation therapy, and prevention and treatment of liver infections are critical to achieve adequate glucometabolic control in TDT patients. Many exciting opportunities remain for further research and therapeutic development. (www.actabiomedica.it)

Key words: Thalassemia; Iron overload; Viral hepatitis; Chronic liver disease; Insulin resistance; Diabetes mellitus

Introduction

β-thalassemia is a global disease that is more prevalent in Southeast Asia, Africa, and Mediterranean countries. However, the global distribution of patients is changing due to population migration, and Northern European countries now have significant thalassemia populations (1).

β-thalassemias have a wide variety of clinical phenotypes that are related to the severity of the β-globin gene mutations prevailing in the population. Based on the severity of clinical phenotype, patients with β-thalassemias are classified in two groups: a) Thalassemia Major (TM) patients who are transfusion-dependent (TDT), and b) Thalassemia Intermedia (TI) patients who are non-transfusion-dependent (NTDT) (1). TDT refers to the patients who require regular blood transfusions for survival since early life and includes patients with severe forms of hematological phenotypes of β-thalassemia e.g., homozygous β^0-thalassemia, β^0/β^+, β/δβ and others. NTDT refers mainly to patients who do not need regular transfusions, but may require occasional transfusion in certain circumstances, such as surgery, pregnancy or infection.

A precise diagnosis requires a comprehensive workup with complete blood count, hemoglobin analysis, and molecular studies to identify the exact genotype of the patient (2-4).

Blood transfusions have greatly improved the survival of TDT patients, while iron chelation therapy has ameliorated the severity and prevalence of iron overload complications, extending further life expectancy. Most major complications are related to treatment and are commonly diagnosed during the second and third decades of life, except for infections which are more prevalent in the first decade of life in some countries (5).

Iron overload, the degree of control of anemia, chronic liver disease (CLD), viral infections and/or genetic factors all play a role in the development of glucose intolerance (6-9).

This review summarizes the fundamental steps of the cell biology and physiology of iron and the current understanding of mechanisms by which iron overload and CLD may induce a deterioration of glucose homeostasis in patients with TDT. Methods for precise assessment of iron overload in clinical practice are also described.

Search strategy

A systematic search was done on December 2020 including Web of Science (ISI), Scopus, PubMed, Embase, and Scholar for papers published in the last 20 years. Moreover, we checked the reference lists of the relevant articles and previously performed reviews for additional pertinent studies. The personal experience on the care of patients with thalassemias is also reported. Conference abstracts, letters, editorials, publications in non-English language and/or brief reports were excluded.

Regulation of iron homeostasis

Iron is an essential mineral required for a variety of molecules to maintain their normal structure and function and for cells to live, grow, and proliferate. The homeostasis of iron results from a tightly coordinated regulation of various proteins involved in uptake, excretion and intracellular storage/trafficking of iron (10).

In a normal individual, 1–2 mg of iron is absorbed daily from the gastrointestinal tract, with an equivalent amount lost by the turnover of gastrointestinal tract epithelial cells. Normally, most stored iron (~1g) is bound by ferritin molecules mainly in hepatocytes, bone marrow, and spleen.

Of these, the liver is considered the primary physiologic source of body iron reserve. The uptake of plasma transferrin-bound iron by the liver is mediated by the cell-surface transferrin receptors (sTfR1 and sTfR2) (11,12). Reticuloendothelial cells store iron as part of the process of phagocytosis and breakdown of aging red cells, extracting iron from heme and returning it to the circulation bound to transferrin. Under normal conditions, the iron saturation of the transferrin is about 30% (11,12). Daily iron recycling accounts for the major part of human iron homeostasis (13,14). Iron homeostasis is strictly regulated, mainly by hepcidin, a hepatic 25-amino-acid peptide which inhibits iron absorption and distribution (11,12).

Iron overload and its complications in transfusion dependent chronic anemias

Iron overload disorders represent a heterogenous group of conditions resulting from inherited and acquired causes. Two main types of acquired iron overload are seen by hematologists, one related to dyserythropoiesis (involving hypo-hepcidinemia), and the other type related to multiple transfusions (thalassemias, aplastic anemia, myelodysplasia, hematopoietic stem cell transplantation) (15). The body lacks a physiologic mechanism of eliminating excess iron (13,14), therefore, in TDT patients and other transfusion-dependent anemias, iron overload occurs in a relatively short time, damaging the liver, heart, pancreas, endocrine glands and other organs (16,17).

Iron toxicity occurs when the amount of circulating Non-Transferrin Bound Iron (NTBI) is increased. The exact chemical nature of NTBI in the plasma is not known, but is thought to consist mainly of ferric citrate and other low-molecular-weight iron species (18,19).

The exact mechanisms of accumulation of iron in particular tissues and the precise toxic effects of iron overload need further systematic studies. However, the formation of the highly toxic hydroxyl radical via the Fenton reaction generates noxious reactive oxygen species (ROS) inducing phospholipid peroxidation, oxidation of amino acid side chains, DNA strain breaks, and protein fragmentation. These can damage lipid membranes, organelles and DNA, causing cell death and fibrogenesis mediated by transforming growth factor 1 (TGF β1) (20). ROS directly activate capsases thereby accelerating apoptotic death (Figure 1). Certain tissues are particularly susceptible to excess iron, for instance, pancreatic b-cells which are also rich in mitochondria and are highly sensitive to oxidant-generating substances (21).

Although several NTBI transporters have been identified as responsible for cellular iron uptake, recent evidence suggests that iron accumulation also depends on iron permeation through the L-type voltage-dependent Ca^{2+} channel (LTCCs) and T-type Ca^{2+} channels (TTCC) (22).

Iron transport through these channels is organ-specific and may explain why the rate of loading observed by serial MRI varies in different organs (liver > pancreas > heart) (Figure 2) (23). The tissues at greatest risk of iron overload due to LTCC activity are the cardiomyocytes, anterior pituitary cells, pancreatic b-cells and neurons. Furthermore, iron clearance differs markedly in the liver and pancreas, leading to a complex relationship between the two parameters.

Other factors contributing to the variability of cellular iron overload are: a) cell surface transferrin receptors and the capacity of the cells to deploy defense mechanisms against inorganic iron; b) individual susceptibility to iron toxic effects; c) the development of organ damage secondary to persisting severe iron overload in the years preceding iron chelation therapy; and d) liver disorders, chronic hypoxia and associated endocrine complications, such as diabetes (24).

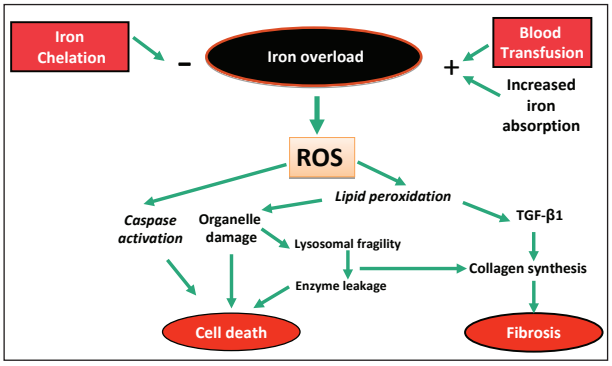


Figure 1. Synthesis from the literature of the pathological pathways and consequences of iron overload. ROS = reactive oxygen species; TGF β1 = transforming growth factor 1 (From: Ref. 20, 21).

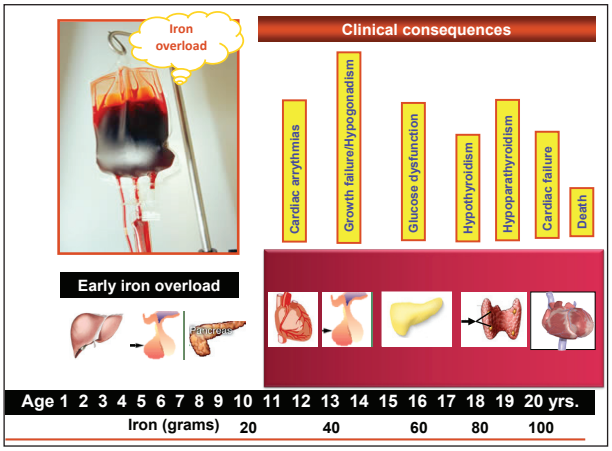


Figure 2. Synthesis from the literature of hypothetical progression of iron overload and development of complications in sub-optimally chelated patients with TDT (From: Ref. 4, 23).

Methods of assessing iron overload

In clinical practice, assessment and monitoring of iron homeostasis include measurement of serum ferritin (SF) and transferrin saturation. They are useful surrogates for total iron stores and extra-hepatic risk, respectively. However, they cannot replace the assessment of liver iron concentration (LIC) or cardiac T2* assessment for monitoring chelator efficacy or stratifying end-organ risk (23).

a. Serum ferritin (SF)

The serum ferritin (SF) pool arises mainly from the liver and reticuloendothelial system. Ferritin is the principal iron storage protein found in the liver, spleen, bone marrow, and in a small amount in the plasma (25). SF values > 1,000 ng/mL indicating iron overload may not correlate closely and in a linear manner

with liver and cardiac iron load. Moreover, this parameter is unreliable in many clinical conditions, such as infections, malignancy, liver disease, vitamin C deficiency and others.

In addition, SF is an acute phase reactant and rises with inflammation. Because of its great variability, guidelines recommend that SF should be measured frequently and only the trend over many measurements should be used for any therapeutic decision-making (25). It seems that efficient chelation therapy, starting at an early age and at SF values persistently < 1,500 ng/mL, may prevent the development of diabetes in TDT patients (4).

b. Liver iron concentration (LIC)

In recent decades, there has been increasing interest in non-invasive iron measurements, especially of the liver and heart, in patients with iron overload. The liver is the main iron storage organ, containing approximately 70% of the total content of the body. The assessment of LIC was previously performed invasively through liver biopsy, but the use of biopsy in clinical practice is limited by the small, but finite, risk of complications, the lack of reproducibility, and sampling errors (25). Nowadays, magnetic resonance imaging (MRI) has replaced liver biopsy as the gold standard for the quantification of LIC given its safety, reliability and reproducibility (23).

Normal LIC levels range between 0.17 and 1.8 mg/g of liver dry weight tissue (mg/g dw) (26).

A LIC value above 3 mg/g dw is considered indicative of liver siderosis, which is classified as mild if the value is < 7 mg/g dw, moderate <15 mg/g dw and severe if >15 mg/g dw (Table 1) (27,28).

In liver MRI, storage iron within tissues influences the magnetic resonance signal by altering the local magnetic field and the signal intensity in both transverse relaxation time (T2) and effective transverse relaxation time (T2*) weighted images

Table 1. Clinical relevance, sensitivity and specificity of liver iron concentrations (mgFe/g dry weight) (From: Ref:27,28).

LIC threshold (1) (mg Fe/g dry weight)	Clinical relevance (1)	Sensitivity (2)	Specificity (2)
1.8	Upper 95% of normal	94% (86–97)	100% (88–100)
3.2	Suggested lower limit of optimal range for LICs for chelation therapy in transfusional iron overload	94% (85–98)	100% (91–100)
7.0	Suggested upper limit of optimal range for LICs for transfusional iron overload and threshold for increased risk of iron-induced complications	89% (79–95)	96% (86–99)
15.0	Threshold for greatly increased risk for cardiac disease and early death in patients with transfusional iron overload	85% (70–94)	92% (83–96)

From: 1. Olivieri NF, Brittenham GM. Blood. 1997;89:739-761. 2. St Pierre TG, et al. Blood. 2005;105:855-661.

Experimental studies of pancreatic b-cell changes in animal models and diabetes risk

Epidemiologic observations in humans and experimental studies in animal models have established a clear association between tissue iron stores and diabetes risk.

The morphologic aspects of iron overload have been studied in a variety of experimental animals, and also in cell cultures (29,30). Rats fed a carbonyl iron-supplemented diet for 4-15 months were studied for iron content and morphologic changes in the liver, spleen, intestinal mucosa, pancreas and heart. All organs had an increased iron content measured by atomic absorption, in a pattern similar to primary human hemochromatosis, with the highest concentrations in the liver and spleen.

Electron microscopic examination of pancreas showed ferritin particles segregated in lysosomes of acinar cells, as well as diffuse cytosiderosis of macrophages in the interstitial septa. In the islets, iron deposits were discrete and present only in b-cells (29-31). Moreover, iron deposition in the interstitial pancreatic cell resulted in excess collagen deposition and defective microcirculation, causing insulin deficiency (32).

Rats and rabbits parenterally treated with a large daily dose of ferric nitrilotriacetate manifested diabetic symptoms after approximately 60 days of treatment. The blood insulin response to oral glucose loading was poor. Heavy iron deposits were found in liver parenchymal cells and in pancreatic exocrine cells. Faint iron staining was found in some pancreatic islet cells, with a reduction in b-granules and weak zinc staining (33).

Furthermore, it has been suggested that the predominance of transferrin receptor expression in pancreatic b-cells results in selective deposition of iron, and predisposes b-cells to damage and diabetes mellitus in iron-overloaded rats (34).

In the hemochromatosis mouse model, iron excess and oxidative stress mediated the apoptosis of pancreatic islets, resulting in a decrease of insulin secretory capacity (35).

Several reports also indicated that islet cells from humans and rodents express low levels of activity of antioxidant proteins, relative to other tissues as the liver, suggesting that islet cells can be easily damaged by oxidative stress (36). Low levels of antioxidant enzyme gene expression may provide an explanation for the sensitivity of pancreatic b-cells towards cytotoxic damage by diabetogenic compounds and during the development of human and animal diabetes.

Finally, current studies suggest that, in streptozotocin-induced diabetic rats, the small peptide hepcidin is directly regulated by insulin which may also play an important role in iron overload (37). Immunohistochemical studies have localized the peptide exclusively to b-cells of the islets of Langerhans and immunoelectron microscopical analyses revealed that hepcidin is confined to the insulin-storing b-cell secretory granules (38). The increased intracellular sequestration of iron and its association with the complications of T2D (39) could be due to a local role of hepcidin in regulating the intrinsic iron homeostasis in different organs.

Studies of pancreatic β-cell changes in humans with iron deposits

Reports of iron loading in the human pancreas from patients with either hemochromatosis or aplastic anemia indicate that iron deposits heavily within the acinar cells of the exocrine pancreas and, to a somewhat lesser degree, in the islets of the endocrine pancreas, as determined by various iron-staining techniques (40-42). Within the islets, iron staining has been determined to be primarily restricted to βcells, with α cells remaining relatively free of iron deposits (40,41). No studies have reported iron accumulation in the other cell populations of the pancreatic islet (e.g. δ cells, ε cells). In patients with aplastic anemia who were receiving blood transfusions, the distribution and the quantity of hemosiderin deposition increased positively in relation to the volume of blood transfused (43).

Pancreatic MRI changes in thalassemic patients and glucose metabolism

Due to the invasive nature of pancreatic biopsies, all data published on human pancreatic iron loading are derived from autopsy. Since MRI can demonstrate

preclinical organ iron deposition in most organs and especially in the liver and heart, it has also been considered a key tool for the assessment of iron overload in other organs, such as pituitary, pancreas, adrenals, spleen, bone marrow, and kidneys (17,44-46).

A number of studies have reported a marked hypointense pancreatic MRI signal in 75-100% of TDT patients (47-52). A strong correlation between pancreatic and hepatic siderosis was reported by Matter et al. (53), although their results have not been confirmed by others (47,48,50). This discrepancy has been attributed to pancreatic fatty replacement in adult patients altering the signal intensity of pancreas. Furthermore, a loss of signal MRI intensity of pancreas was accelerated after splenectomy (53,54). Thus, splenectomized TDT patients should be strictly monitored for pancreatic iron overload by MRI to detect early pancreatic alterations (53). A normal pancreas T2* value showed a 100% negative predictive value for disturbances of glucose metabolism and for cardiac iron overload (54).

Pepe et al. (55) systematically explored the link of pancreatic iron with glucose metabolism and with cardiac complications in a cohort of 1,079 TDT patients. Lower pancreas T2* values were associated with cardiac disease. Patients with normal glucose metabolism showed significantly higher global pancreas T2* values than patients with impaired fasting glucose, impaired glucose tolerance or diabetes. A mean pancreatic MRI T2* relaxation time <13.07 ms predicted an abnormal OGTT, while a lower cut-off value of 5.6 ms was reported by Kosaryan et al. (56).

More recently, Shur et al. (57) retrospectively evaluated the pancreatic and hepatic R2* (R2* =1/T2*), fat fraction (FF), LIC, and glucose metabolism in 105 TDT patients with iron overload. There were no significant differences in pancreatic R2*, liver R2*, and FF in patients with iron overload and glucose dysregulation compared to those with normoglycemia. However, the pancreatic FF was significantly higher in TDT patients with dysglycemia (23.5%) compared to those with normoglycemia (16.7%, p = 0.011). The authors concluded that simultaneous measurements of fat and iron are essential to avoid confounding effects during quantitative analysis. In other reports, a weak or moderate correlation was found between T2* of pancreas and LIC (50,56,58).

Liver iron, hepatitis C and diabetes in thalassemia

Liver complications in thalassemias are due to several factors, dominated by chronic iron overload and chronic viral infections (59). Hepatitis B virus (HBV) or Hepatitis C (HCV), and possible infections with more than one HCV genotype through blood transfusions during the past decades, have contributed to liver fibrosis and liver carcinoma (60-63). In 1990 the reported prevalence of HCV infection after HCV antibody testing in TDT patients were: 32.3% for U.K, 34% for France, 40-50% for Greece and 72.2% for Italy (60). The wide variation in the prevalence was attributed to differences in the prevalence of HCV infection in blood donors, in the age distribution of patients, and the number of blood units transfused (64).

Insulin resistance (IR) is common in patients with chronic hepatitis C, even with minimal fibrosis, compared to healthy controls, and is associated with increased liver iron deposits and glucose abnormalities (65). The interplay between liver siderosis and HCV infection facilitates the progression to diabetes mellitus, at least in adulthood. This potential effect seems to be related to different HCV genotypes (66,67). Genotypes 1 and 4 and high serum HCV-RNA quantitative measurement of viral load have been reported to be associated with more severe IR (66-68).

Zinc, vitamin D and glucose homeostasis in thalassemia

Low serum zinc (Zn) levels and vitamin D deficiency (VDD) are common in patients with TDT and may contribute to deterioration of glucose homeostasis (69-72). Zn participates as a potent physiological regulator of insulin signal transduction through its inhibitory effect on protein tyrosine phosphatase 1b, the key phosphatase that dephosphorylates the insulin receptor (73,74).

Zinc deficiency might lead to an exacerbation of the inability of the pancreas to secrete sufficient amounts of insulin in response to glucose stimulation in TDT patients, suggesting that serum zinc levels should be routinely monitored in these patients, as it might provide useful complementary information re-

garding glucose metabolism (69). The potential role of vitamin D deficiency in IR has been proposed to be associated with inherited gene polymorphisms including vitamin D-binding protein, vitamin D receptor, and vitamin D 1 alpha-hydroxylase gene (75,76).

A meta-analysis of randomized controlled trials (RCTs) found that vitamin D supplementation (4000 IU/d. with a median trial duration of 4 months) significantly reduced fasting glucose by 0.11 mmol/L, fasting insulin by 1.47 mIU/L, and Homeostasis Model Assessment of insulin resistance (HOMA-IR) by 0.32 (77). Future well-designed trials are warranted to confirm these findings and validate optimal vitamin D dosage.

Prevalence, risk factors and importance of early diagnosis of glucose abnormalities in thalassemia

a. Prevalence

A link between iron overload and the development of diabetes dates to the initial case report of Bannerman et al. in 1967 (78). In the following years, several publications have described the prevalence of diabetes in thalassemia patients in various countries. In 1995, a multicentre Italian study, involving 1,861 TDT patients, showed that the overall rate of DM was 4.9% (mean age 18.1 years) (79). Nine years later, a questionnaire was sent to 29 Centres treating a total of 3,817 TDT patients, with 36% of those being over the age of 16 years. Impaired glucose tolerance (IGT) was observed in 6.5% of patients and DM in 3.2%. The mean age at diagnosis of impaired glucose metabolism was 17 years in both groups of TDT patients. Compliance to chelation therapy was poor in 51% (mean SF levels in IGT patients: 4,545 ng/mL; in DM patients: 3,585 ng/ml) and serum liver enzymes were reported "high" (2-3 times the normal levels) in 65% of the entire group (80).

More recently, in a tertiary adult thalassemia unit in the UK, Ang et al.(81) showed that 41% of 92 TDT patients (median age 36 yrs) had DM which was associated with a mean SF >2,000 ng/mL.

In 2019, He et al.(82) conducted a meta-analysis on the prevalence of abnormal glucose metabolism in TDT patients, including a total of 35 studies from 1994 to 2018. The overall prevalence of DM was 6.5% (95% CI: 5.3 %-7.7 %) and had gradually increased over the period of the study. Seven studies examined the prevalence of impaired fasting glucose (IFG) and twelve, impaired glucose tolerance (IGT), reporting an overall prevalence of 17.2% (95% CI: 8.4%-26.0%) and 12.4% (95% CI: 5.9%-18.9%), respectively. The highest prevalence of IFG was reported in the Middle East (27.8%; 95% CI: 18.5%-37.2%) and the highest prevalence of IGT was registered in the Mediterranean coast (15.1%; 95% CI: 3.5%-26.7%) (82).

Several limitations were reported by the authors in their study; significant heterogeneity was found in the pooled analyses of prevalence, probably due to differences in the number of cases or basic patients' characteristics, gender, age at diagnosis of endocrinopathy, SF levels, length of blood transfusion and percentage rates of glucose abnormalities in younger patients.

b. The importance of an early diagnosis of dysglycemia

Taking into consideration that pancreatic iron loading starts in early childhood in patients receiving suboptimal iron chelation (53,83-85), we selected two studies from the literature to analyze in detail the reported precocity of glucose dysregulation in children and adolescents with TD.

In sixty-seven Indian patients with TDT (mean age: 7.4 ± 4.4 years, range: 1 - 20 years), 8 (11.9%) had impaired fasting glucose (IFG), 7 (10.4%) had IGT, and 1 (1.4%) had DM. Patients with abnormal glucose profile had longer disease duration, higher fasting BG and SF compared with normoglycemic subjects. Patients on deferiprone alone significantly improved glucose homeostasis on follow-up than those on desferrioxamine or combination therapy of desferrioxamine and deferiprone (P: <0.05) (86).

In a prospective, case-controlled, observational study, Liang et al. (87) enrolled 267 Chinese children with TDT with iron overload (mean age: 7.9 ± 0.2 years; 60% males; SF: 4,476 ± 158 ng/mL; serum ALT: 52.0 ± 2.6 IU/L) and 80 healthy controls (mean age: 10.3 ± 0.3 years; 38% males). All TDT were receiving blood transfusions and were chelated either by monotherapy (desferrioxamine, deferasirox, or deferiprone) or combination therapy according to their body weight, degree of iron overload and tolerance.

In the TDT group, 81 (30%) had IFG and five (2%) had diabetes. The stepwise logistic regression analysis showed that an age of >10 years, a SF level > 2,500 ng/mL, a cardiac T2* < 20 ms, and serum ALT >50 IU/L had independent effects on the abnormal glucose metabolism. Children over 10 years of age had a threefold higher risk of development of IFG or DM. However, most children in this cohort, despite being on a transfusion programme from a very early age, were poorly transfused and suboptimally chelated due to chronic shortage of blood and scarce resources within the health insurance.

The prevalence of glucose metabolism dysregulation found in this study was much higher compared to the Metwalley et al.(88) report (13%) on 60 thalassaemic children.

In brief, the wide variation in prevalence of glucose dysregulation in TDT patients is multifactorial. It is affected by the duration of blood transfusion, the annual blood consumption, the degree of iron load, organ dysfunction, type of chelators and compliance to therapy and age of patient. Prolonged disease duration, higher SF and ALT are associated with impaired glucose homeostasis in thalassemic children and adolescents.

c. Etiology and pathogenesis of glucose abnormalities

In patients receiving suboptimal iron chelation, pancreatic iron loading starts in early childhood.

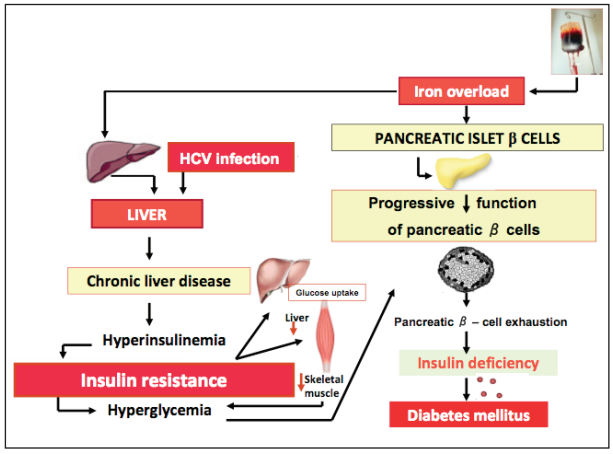


Figure 3. Pathogenesis of mechanisms inducing glycemic abnormalities in transfusion-dependent β-thalassemia TDT patients.TDT patients with and without diabetes mellitus.among the 100 top cited articles

With advancing age, a persistent IR along with the decrease in the circulating insulin levels (due to declining β-cell function), leads to the onset of glucose intolerance that progresses to frank DM (Figure 3). Pancreatic autoimmunity (89), Zn deficiency and VDD (69-72) are other factors that can affect pancreatic function. Overall, the cumulative effects of these diabetogenic factors can lead to glucose intolerance as a long-term progressive result (90).

Several studies have shown that IR and insulin deficiency mark both the prediabetic state and diabetes in thalassemia (91-93). IR probably occurs at the level of the liver (due to hepatic iron load and liver dysfunction), where it might interfere with insulin's ability to suppress hepatic glucose uptake, and also at the level of the muscle, where iron deposits might decrease the glucose uptake. Insulin secretory defects may originate from pancreatic β-cell iron toxicity damage rather than from IR (94). Moreover, an acute effect of blood transfusion on insulin sensitivity and β-cell function has been reported by Wankanit et al. (95). The authors speculated that an increase of serum ferritin and Hb following blood transfusion may contribute to an increase in insulin secretion and thus to a trend towards increased IR.

Pancreatic islets have an extreme susceptibility to oxidative stress, perhaps because of the nearly exclusive reliance on mitochondrial metabolism of glucose for glucose-induced insulin secretion (24,96).

It is possible that in patients with both iron overload and diabetes a diminished pancreatic mass is the result, rather than the cause, of decreased β cell function as insulin is thought to promote acinar cell growth and a lack of insulin is associated with decreased pancreatic mass (97). Fatty degeneration of the pancreas could be an additional risk factor for the development of diabetes in TDT patients, but this hypothesis needs further studies (98).

d. Iron chelation therapy

Iron chelators in current clinical use include monotherapy with DFO, oral deferiprone (DFP), or oral deferasirox (DFX), and combination therapy with subcutaneous DFO and oral DFP. Many studies have been conducted to assess the efficacy and safety of the three iron chelators. Monotherapy is generally utilized

if the iron burden is at acceptable or near-acceptable levels and the dose is adjusted accordingly. DFO has the advantage of a long history of use in terms of safety and efficacy; however, the adherence to therapy is a challenge. Combination chelation is often employed for patients with high iron burden, iron-related organ injury, or where adverse effects of chelators preclude administration of an appropriate chelator dose (99). Optimal dosing, side-effect monitoring, and adherence to chelation are essential for any iron chelator. Other agents that aim to reduce oxidative stress and iron overload (hepcidin agonists, erythroferrone inhibitors and exogenous transferrin) are under experimental investigation (100).

Irrespective of the chelation agent, adherence to iron chelation therapy is essential for prevention of dysglycemia and has been shown to improve glucose intolerance (87,101-107).

The personal experience in Ferrara

The records of 273 patients with thalassaemia major followed in the Ferrara Centre between 1954 and 2004 were reviewed (108). The patients' mean age at the appearance of DM in the eighties was 18.2 ± 3.6 years. At the diagnosis of DM, SF levels were available in 26 patients (3,847 ± 2,282 ng/mL, range: 655 - 9,000). The SF levels were below 1,500 ng/mL in 4 patients and higher in the remaining 23 patients (Figure 4) and the serum alanine aminotransferase (ALT) levels were increased in 95% of patients. Endocrine and cardiac complications were more common in patients with DM than in non-diabetic patients (Figure 5). Poor compliance with subcutaneous desferrioxamine (DFO) therapy, interval between the age at first transfusion and the age at the start of subcutaneous chelation therapy with DFO, liver cirrhosis or CLD with severe fibrosis were significantly associated with the presence of DM (Figure 6). Over the years, the frequency of DM has gradually decreased and the age of onset of DM progressively increased. This is probably due to a better compliance to iron chelation therapy with desferrioxamine (DFO) and to the availability of new oral chelators. From 2004 to 2020, a further five cases (4 females) of DM were documented (T2D in two of them).

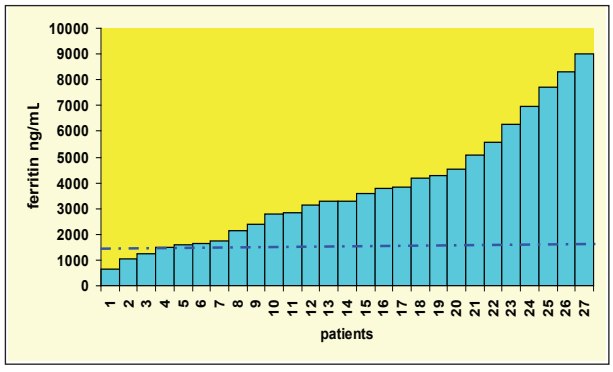
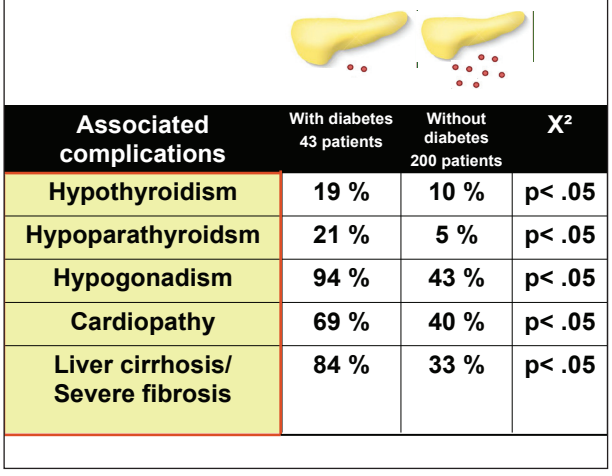
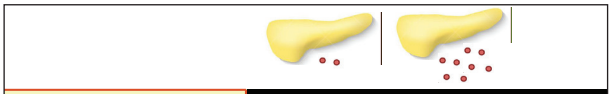


Figure 4. Serum ferritin levels in TDT patients at the diagnosis of diabetes mellitus.



Associated complications	With diabetes 43 patients	Without diabetes 200 patients	X^2
Hypothyroidism	19 %	10 %	p< .05
Hypoparathyroidsm	21 %	5 %	p< .05
Hypogonadism	94 %	43 %	p< .05
Cardiopathy	69 %	40 %	p< .05
Liver cirrhosis/ Severe fibrosis	84 %	33 %	p< .05

Figure 5. Associated complications related to iron overload in TDT patients with and without diabetes mellitus.among the 100 top cited articles



Variables	With diabetes mellitus	Without diabetes mellitus	X^2
Positive family history	50 %	38 %	ns
Sex (F: M)	24/19	54/142	ns
Age 1st transfusion (yrs)	1,7 ± 1,8	1,4 ± 1,5	ns
Splenectomy	86 %	79 %	ns
Age at splenectomy (yrs)	10 ± 6	10 ± 7	ns
Age (yrs) at starting desferrioxamine s.c.	10 ± 4	7 ± 5	P<.05
Compliance with DFO (6/7 days)	16 %	42 %	P<.001
Liver cirrhosis/ severe fibrosis	84 %	33 %	P<.0001

Figure 6. Risk factors for diabetes mellitus in TDT patients.

Conclusion

Understanding the sequence of abnormalities in the progression from normal glucose homeostasis to DM and identifying the risk factors for the glycometabolic defects in thalassemic patients might help in the formulation of interventions. Iron overload and chronic hepatitis C could play a role in IR. Currently, the relative contributions between systemic IR and declining β cell function to the development of diabetes in patients with thalassemia are still undefined.

Traditionally, iron overload has been assessed by serial serum ferritin measurements and liver biopsies. Recent advances in the assessment of iron burden in thalassemia patients enable closer monitoring of organ-specific iron stores with more personalized adjustments in chelation regimens.

Recently, cardiac, liver and pancreatic iron load measurement by MRI represent an efficient tool to manage individual organ iron stores and to optimize iron chelation therapy. In patients receiving suboptimal iron chelation, pancreatic iron loading starts during early childhood. With advancing age, persistence of IR along with declining β-cell function leads to glucose intolerance and frank DM.

The presence of HCV infection in TDT patients may also be an additional risk factor for the development of IGT through the induction of IR.

In conclusion, the complexity of glucose disturbances in patients with TDT requires expertise of healthcare professionals and a multidisciplinary approach in order to ensure rigorous monitoring and adequate control of glucose metabolism. A regular packed red blood cells (PRBCs) transfusional program, a close and early diagnosis of iron overload and monitoring of appropriate iron chelation therapy, combined with prevention of liver infections, are critical measures to achieve efficient disease control.

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