

Iron overload in transfusion-dependent survivors of hemoglobin Bart's hydrops fetalis.

Homozygous α^0 -thalassemia (hemoglobin Bart's hydrops fetalis) results from deletion of all duplicated α -globin genes on chromosome 16p. The absent production of α -globins leads to significant fetal anemia and hypoxia, and subsequently hydrops fetalis. Without intrauterine transfusion, almost all affected patients die in-utero or shortly after birth.¹ Similar to patients with

transfusion-dependent beta-thalassemia (TDT- β), survivors of hemoglobin Bart's hydrops fetalis require life-long regular transfusions.¹ Without effective iron-chelation therapy, frequent transfusions of iron-rich erythrocytes ultimately results in saturation of transferrin and generation of toxic non-transferrin bound iron (NTBI). NTBI is then deposited in organs, mainly the liver, heart, and endocrine system with eventual organ dysfunction.² In chronically transfused patients with TDT- β , iron loading is predominantly derived from blood transfusions.³ By contrast, dysregulation of hepcidin, the main regulator

Table 1. Patient characteristics of the study population.

	α -Thalassemia	β -Thalassemia
Demographics		
Number of Patients	7 ¹	14
Male, N (%)	5 (71.4)	8 (57.1)
Number of assessments, N, (%)	54	79
Type of mutations		
--SEA/--SEA	6	–
--SEA/--FIL	1	–
β^0/β^0	–	7
β^0/β^+	–	4
β^+/β^+	–	2
β^+/β^+ and $\alpha\alpha\alpha/\alpha\alpha$	–	1
Longitudinal Variables		
Age at assessments [year], Mean (SD)	7.38 (4.73)	11.13 (4.67)
Method of assessment, N (%)		
Biopsy	23 (42.6%)	20 (25.3%)
FerriScan	31 (57.4%)	59 (74.7%)
Type of chelation medication at each assessment. N (%)		
Deferoxamine	39 (72.2)	25 (31.6)
Deferasirox	15 (27.8)	47 (59.5)
Not on chelation	0	7 (8.9)
Longitudinal Outcomes		
LIC [mg/g], Mean (SD)	9.68 (5.73)	8.63 (7.99)
Ferritin [g/L], Mean (SD)	849.33 (573.54)	2036.39 (1495.92)
Ferritin/LIC [mg/L], Mean (SD)	86.87 (33.22)	308.58 (182.43)
Other variables (not included in the model)		
Pre-transfusion hemoglobin (g/L)	99 (7)	95 (6)
Serum iron/transferrin [micromol/g], Mean (SD) * ²	1.79 (0.32)	2.48 (0.65)
Reticulocyte count [$\times 10^9/L$], Mean (SD) * ²	613 (82)	<20
Ferritin at 12 months of age [g/dL], Mean (SD) * ^{3,4}	1242.4 (419.4)	418.3 (113.3)
Age of initiation of chelation [Month], Mean (SD) *	16.3 (3.2)	34.2 (8.1)
Age of chelation switch from deferoxamine to deferasirox [Year], Mean (SD) ⁵	9.8 (2.7)	9.2 (3.6)
Cardiac T2* [ms] ⁶	38.6 (3.2)	36.2 (2.5)

These data are for homozygous α^0 -thalassemia patients and TDT β patients who were treated on a unified protocol to keep the pre-transfusion hemoglobin >90 g/L and LIC <5 mg/g of dry-liver-weight. Corresponding pairs of serum ferritins and LIC assessments (defined as measurements of not more than two weeks apart with no transfusions in between) were used to calculate ferritin-to-LIC ratios. The higher proportion of liver biopsies in alpha-thalassemia patients reflects the assessment of iron in these patients at a younger age and prior to the establishment of FerriScan as the mode of LIC assessment in our institution. Method of LIC assessment was included in the model as the assessment method could have confounded the effect of age in a longitudinal data analysis and discrepancies between results of method of LIC assessment have previously been reported.¹⁴ 1: data of the two patients who were born after the implementation of the aggressive transfusion strategy are not included in this table. 2: presented data are for the latest year on conventional transfusions. 3: data not available for 1 patient with homozygous α^0 -thalassemia and 3 patients with TDT β . 4: no patient was on iron chelation at the time of ferritin assessment at 12 months of age. Note that ferritin was lower in homozygous α^0 -thalassemia group when on iron chelation, as chelation in all patients was titrated to target a LIC of <5 mg/g of dry-liver-weight, but in chelation-naïve patients, ferritin was significantly higher in homozygous α^0 -thalassemia group. 5: All patients were initially started on deferoxamine and patients older than 6 years were eventually switched to deferasirox. Please note that deferiprone had not been approved in Canada during the period of this study. 6: Only 4 patients with homozygous α^0 -thalassemia and 9 patients with TDT β were older than 10 years and had assessments of cardiac iron. Given the limited number of evaluations, the absence of any statistical significance should be interpreted with caution. *Statistically significant (for variables not included in the multivariate analysis. See *Online Supplementary File* for more details).

of iron homeostasis, drives the iron overload in patients with iron loading anemias (e.g., non-transfusion-dependent thalassemia [NTDT]).^{4,5}

We have recently reported that the underlying disease process in homozygous α^0 -thalassemia differs from that of TDT- β .⁶ The production of non-functional hemoglobin-H (HbH) and the resulting tissue hypoxia play a central role in the pathophysiology of homozygous α^0 -thalassemia. When regularly transfused using TDT- β protocols, patients with homozygous α^0 -thalassemia still show features of hypoxia and erythropoietin-driven increased erythropoietic activity, including marked reticulocytosis and increased soluble transferrin receptor, while erythropoiesis is generally suppressed in adequately transfused β -thalassemia patients.⁵ A more aggressive transfusion regimen aimed at achieving optimal “functional” hemoglobin concentration and improved tissue oxygenation resulted in decreased serum erythropoietin levels and reduced erythropoietic activity in patients with homozygous α^0 -thalassemia.⁶ On the backdrop of these pathophysiological differences as outlined in our previous report, we hereby present our data on iron overload in patients with homozygous α^0 -thalassemia. We show that the pattern of iron overload in these patients also differs from their TDT- β counterparts, suggesting that both transfusional iron loading and increased intestinal absorption of iron contribute to siderosis in these patients.

Following Institutional Review Board approval, we retrospectively collected longitudinal data on a birth cohort of 9 patients with homozygous α^0 -thalassemia and 14 other patients with TDT- β followed at the Hospital for Sick Children and McMaster Children’s Hospital. None of the patients had been splenectomized. All homozygous α^0 -thalassemia patients received intrauterine transfusions followed by regular chronic transfusions after birth. Up until 2014, seven of these patients (all born before 2009) were on a transfusion program targeting a pre-transfusion total hemoglobin of >90 g/L, similar to TDT- β protocols. The transfusion regimen was later intensified in four of these patients targeting a pre-transfusion “functional” hemoglobin (non-HbH) of >100 g/L

with a goal to suppress the markedly increased erythropoiesis and improve tissue oxygenation.⁶ Of the three who were not aggressively transfused, one received hematopoietic stem cell transplant at age 5 years and no longer required transfusions, one had transferred to another institution for adult care, and one remained on conventional transfusion regimen. Two additional patients were born after 2014 and have been on the aggressive transfusion regimen since birth.

Collected data included patients’ demographics, age at each assessment, annual liver iron concentration (LIC), method of LIC assessment [liver biopsy or R2-MRI (FerriScan®)], cardiac T2* MRIs, type of chelation medication, serum iron, transferrin and ferritin measurements, peripheral blood reticulocyte count as well as other biochemical and hematological parameters (Table 1). We used linear mixed models to assess the associations between ferritin, LIC and ferritin-to-LIC ratio with variables of interest including age, chelation medication, disease type, and method of LIC assessment in seven homozygous α^0 -thalassemia and 14 TDT- β patients who have been transfused with the same protocol targeting total Hb >90 g/L and LIC <5 mg/g dry-liver-weight. Linear mixed model effectively accounted for the correlations within a subject and between different ages. We also assessed the association between peripheral blood reticulocyte count and ferritin-to-LIC ratio in homozygous α^0 -thalassemia patients. We used the Kruskal-Wallis test to assess the effect of the aggressive transfusion strategy on ferritin-to-LIC and iron-to-transferrin ratios in the homozygous α^0 -thalassemia patients.

Table 1 summarizes the patient characteristics before the implementation of the aggressive transfusion strategy. At 12 months of age, patients with homozygous α^0 -thalassemia had significantly higher serum ferritin indicating earlier iron overload from earlier initiation of transfusions compared to their TDT- β counterparts (Table 1).

In both patients with homozygous α^0 -thalassemia and TDT- β , ferritin was significantly correlated with LIC (Figure 1). Univariate and multivariable analyses showed

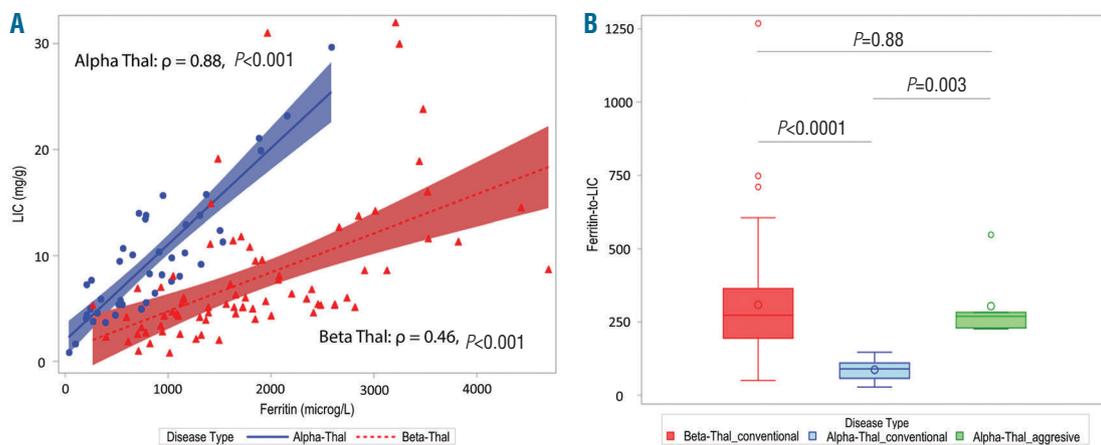


Figure 1. Correlation of ferritin and liver iron concentration (LIC) in study patients. (A) Scatter plot showing the association of ferritin with LIC in homozygous α^0 -thalassemia and transfusion-dependent β -thalassemia patients who have been transfused with the same protocol (conventional protocol) to keep total hemoglobin >90 g/L. (B) Comparison of ferritin-to-LIC in 14 transfusion-dependent β -thalassemia (red) vs. 4 homozygous α^0 -thalassemia, before (blue, Alpha-Thal_conventional) and after (green, Alpha-Thal_aggressive) implementation of aggressive transfusion strategy which targeted a pre-transfusion “functional” (non-HbH) hemoglobin of >100 g/L. Compared to transfusion-dependent β -thalassemia patients, ferritin-to-LIC was significantly lower in homozygous α^0 -thalassemia patients on conventional transfusion strategy but not after implementation of aggressive transfusion in these patients.

Table 2. Hematologic and biochemical changes with aggressive transfusion in four homozygous α^0 -thalassemia patients.

	Conventional transfusion Mean (SD)	Aggressive transfusion Mean (SD)	P
Pre-transfusion hemoglobin (g/L)	101 (7)	118 (6)	<0.001
Hemoglobin H (%)	41.9 (11.3)	19 (3)	<0.001
Calculated functional hemoglobin (g/L)	58 (12)	95 (6)	<0.001
Annual transfusion volume (cc/kg/year)	208 (14)	265 (21)	<0.001
Calculated transfusional iron loading rate (mg/kg/day) ¹	0.40 (0.02)	0.51 (0.04)	<0.001
Serum ferritin-to-LIC ratio (mg/L)	86.8 (33.2)	304.2 (121.5)	0.003
Serum erythropoietin (mU/mL)	362.6 (272.1)	47.6 (32.3)	<0.001
Soluble transferrin receptor (mg/L)	7.31 (0.91)	2.15 (0.58)	<0.001
Reticulocyte count (x10 ⁹ /L)	631 (82)	354 (36)	<0.001
Serum bilirubin (micromol/L)	57.3 (10.4)	22.0 (5.5)	<0.001
Serum iron-to-transferrin ratio (micromol/g)	1.83 (0.27)	2.21 (0.19)	0.003

¹Calculated as per Trompeter and Cohen,¹⁵ and based on an average hematocrit of 65%.¹⁶ Similar results were obtained when data of three patients who remained on conventional transfusion and two patients who have been transfused with aggressive transfusion since birth were included in the analysis (*detailed data not shown*).

no difference in LIC between the two groups ($P=0.35$), but ferritin was found to be significantly lower in homozygous α^0 -thalassemia patients ($P=0.0013$). When adjusted for other confounding variables including chelation medication and method of LIC assessment, which were both predictors of ferritin-to-LIC ratios ($P=0.028$ and 0.027 respectively), the disease type was found to be an independent and highly significant predictor of ferritin-to-LIC ratio; homozygous α^0 -thalassemia patients had significantly lower ferritin-to-LIC ratios ($P<0.0001$) (see *Online Supplementary File* for more detailed results). We also observed a trend, although not statistically significant, towards lower ferritin-to-LIC ratio in the older patients with homozygous α^0 -thalassemia ($P=0.07$, $\rho=-0.28$). In addition, reticulocyte count was correlated with ferritin-to-LIC ratio ($P=0.042$, $\rho=0.41$) in homozygous α^0 -thalassemia patients.

We further studied the ferritin-to-LIC ratio and iron-to-transferrin ratio (as a surrogate measure for unbound iron) in the homozygous α^0 -thalassemia patients whose transfusion regimen was switched from a conservative approach to a hyper-transfusion regimen including exchange blood transfusion. Both serum ferritin-to-LIC and iron-to-transferrin ratios were now significantly higher, and were similar to ratios in the conventionally transfused TDT- β patients (Figure 1, Table 2).

While homozygous α^0 -thalassemia is currently considered a rare disease, its prevalence is likely to rise given the high α^0 -thalassemia gene carrier rates in Southeast Asia coupled with recent advances in fetal medicine that is delivering improved access to intra-uterine transfusions.¹⁷ Defining the pathophysiologic processes unique to this patient population, including the mechanism of iron overload, therefore, assumes greater relevance and significance.

In this study, we have demonstrated important differences in the pattern of iron overload between patients with homozygous α^0 -thalassemia and those with TDT- β . As expected, patients with homozygous α^0 -thalassemia had earlier iron overload as their transfusions had started in-utero. Furthermore, we have shown that in regularly transfused homozygous α^0 -thalassemia patients, serum ferritin can significantly underestimate LIC and bears similarity with a wide range of NTDT syndromes.⁸⁻¹²

While we did not directly measure the hepcidin in this retrospective study, the lower ferritin-to-LIC ratios observed in our homozygous α^0 -thalassemia patients indirectly suggest that increased gastrointestinal absorption of iron due to suppressed hepcidin may contribute to siderosis in these patients, in addition to transfusional iron loading. It has been proposed that in patients with transfusion-associated iron overload, transfused iron is initially distributed in the reticuloendothelial system accounting for the proportionally higher plasma ferritin levels, in contrast to iron loading disorders, which are driven by hepcidin suppression.⁸⁻¹² A likely explanation is that in homozygous α^0 -thalassemia patients who are transfused according to TDT- β protocols, the high level of circulating non-functional HbH leads to poor tissue oxygen delivery and hypoxia-driven increase in serum erythropoietin and erythropoietic activity,⁶ which drive erythroferrone-mediated hepcidin suppression. In support of this hypothesis, we also observed an association between serum ferritin-to-LIC ratios with reticulocyte count in homozygous α^0 -thalassemia patients. Furthermore, the switch to a more aggressive transfusion regimen decreased serum erythropoietin and suppressed erythropoietic activity accounting for the observed increase in serum ferritin-to-LIC ratios.⁶ Our patients continued to have reticulocytosis even while on an aggressive transfusion, suggesting that hypertransfusion did not completely normalize erythropoietic activity and thus may not have completely blunted gastrointestinal absorption of iron in these patients during the period of this study.

Our study has limitations in being retrospectively designed and involving a small number of patients. We have attempted to mitigate their impact by analyzing carefully-collected longitudinal data in a cohort of patients treated with the same transfusion regimen. Our data provide new insights into the pattern of iron overload in patients with homozygous α^0 -thalassemia. Our data suggest that a hypertransfusion regimen using exchange transfusions might provide an avenue to effectively reduce HbH while reducing the net transfusional iron loading associated with this approach. Further elucidation of the exact mechanism of iron overload involving direct measures of iron homeostasis such as hepcidin and

NTBI can help guide the development of novel therapeutic approaches (e.g., therapies that target the hepcidin-ferroportin system) to optimize treatment of iron overload in this unique patient population.¹³

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