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## Correlation of liver iron concentration determined by R2 magnetic resonance imaging with serum ferritin in patients with thalassemia intermedia

Thalassemia intermedia is a highly diverse group of thalassemia syndromes associated with anemia and a range of specific complications, such as extramedullary hematopoiesis, leg ulcers, gallstones and a hypercoagulable state, which are uncommon in patients with thalassemia major.[1] The degree of anemia present in patients with thalassemia intermedia is typically mild and generally does not require regular blood transfusion therapy. However, patients can still be at risk of the clinical sequelae of iron overload (as commonly seen in regularly transfused thalassemia major patients) due to increased intestinal iron absorption triggered by chronic anemia, ineffective erythropoiesis and, possibly, decreased serum hepcidin.[2,3] The principal methods of determining body iron levels are measurement of serum ferritin levels and assessment of liver iron concentration from biopsy tissue. Non-invasive approaches for determining liver iron concentration are increasingly used as an alternative to biopsy, although R2 magnetic resonance imaging (MRI) is currently the only validated approach.[4,5] A significant correlation between serum ferritin and liver iron concentration has been established in regularly transfused patients with thalassemia major.[6,7] Data of patients with thalassemia intermedia are limited, but recent studies have highlighted differences compared with the studies performed in thalassemia major patients.[3,8] In these studies, serum ferritin levels were seen to be significantly lower in patients with thalassaemia intermedia than in those with thalassemia major, despite comparable liver iron concentration (as evaluated by biopsy or superconducting quantum interference device). The aim of our study was to investigate the correlation between liver iron concentration determined by R2 MRI and serum ferritin levels in patients with thalassemia intermedia. The data reported here represent the largest investigation of this correlation in thalassemia intermedia using R2 MRI and, therefore, provide valuable information on the relationship between these parameters in this specific patient population.

This was a cross-sectional study of randomly selected thalassemia intermedia patients treated at a chronic care center in Hazmieh, Lebanon. The sampling frame consisted of 120 thalassemia intermedia patients ≥2 years of age. We were able to contact 109 of these patients by telephone and 74 agreed to participate. Patient charts were reviewed and a medical history compiled, which included details of drug history, co-morbid illnesses and transfusional history. Data from a randomly selected population of patients with thalassemia major treated at the center were also obtained for comparative evaluation. Blood samples were obtained for assessment of serum ferritin levels. Direct determination of iron burden was performed using R2 MRI to obtain liver iron concentration values, using established methodology.[9] The reading of MRI results was performed by Dr. Tim St Pierre. Written informed consent was provided by all patients. Data from 74 thalassemia intermedia patients were included in the analysis (Table 1). Transfusion-naïve patients had significantly lower iron levels compared to those with a history of transfusion therapy ($p$=0.003). None of the patients were receiving iron chelation therapy at the time of data collection and had not received chelation therapy for at least two years prior to study entry. In addition, none of the patients involved were

**Table 1.** Patients' characteristics.

| | *Patients' characteristics* |
|---|---|
| Patient number | n=74 |
| Mean age, ±SD, in years (range) | 26.5±11.5 (8-54) |
| Male/female | 33/41 |
| Splenectomized, (%) | 59 (79.7) |
| Mean hemoglobin, g/dL±SD (range) | 8.43±1.86 (4.90-13.10) |
| Transfusion history | |
| Naïve | 20 |
| Transfused | 54 |
| Mean SF±SD, ng/mL (range) | 1023±780 (15-4140) |
| Splenectomized | 1201±764 |
| Non-splenectomized | 428±495 |
| Transfusion-naïve | 567.8±455.2 |
| Transfused | 1209±429 |
| Mean LIC±SD, mg Fe/g dw (range) | 9.0±7.4 (0.5-32.1) |
| Splenectomized | 10.5±6.8 |
| Non-splenectomized | 3.9±7.4 |
| Transfusion-naïve | 4.0±3.3 |
| Transfused | 11.55±7.00 |

*SF: serum ferritin; LIC: liver iron concentration; dw: dry weight.*

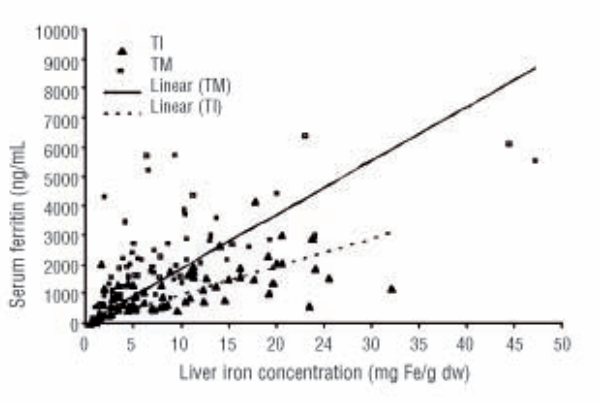


**Figure 1.** Serum ferritin versus liver iron concentration as determined by R2 MRI (line equation for thalassemia intermedia: serum ferritin = 67.8*liver iron concentration; thalassemia major: serum ferritin=106.6*liver iron concentration).

positive for hepatitis B or C virus or had elevated alanine aminotransferase levels. Iron levels in the study population demonstrate that many patients with thalassemia intermedia have serum ferritin and liver iron concentration levels above the recommended threshold levels identified in patients with thalassemia major, indicating a risk of significant morbidity and mortality. A positive correlation between age and serum ferritin levels (R=0.29; $p$=0.01), and between age and liver iron concentration (R=0.35; $p$=0.002) was observed. After standardizing for serum ferritin level, a near significant correlation between age and liver iron concentration was observed (R=0.23; $p$=0.055). Serum ferritin levels were, therefore, seen to increase with age, reflecting increased iron accumulation over time, even in the absence of transfusion therapy. While this observation is in accordance with one previous study,[10] others have not reported increasing iron levels.[8,11] This highlights the variability in iron loading in patients with thalassemia intermedia and the need for a more accurate assessment of iron burden in these patients.

A significant positive correlation between mean serum ferritin and liver iron concentration values was observed in our study (R=0.64; $p$<0.001). Previous observations are limited, but two studies have been performed, one demonstrating a statistically significant correlation,[8] the other showing no significant correlation.[3] The relationship between liver iron concentration and serum ferritin has previously been shown to lack significance in splenectomized thalassemia intermedia patients,[3] but data from our study show a positive correlation in this subset (R=0.62; $p$<0.001), thereby supporting the use of liver iron concentration assessment in splenectomized thalassemia intermedia patients.

Comparative data from the patients with thalassemia major (n=65; 36 male, 29 female; mean age 18.35 [SD ± 7.33; range 7–44]) showed mean liver iron concentration values of 9.2 mg Fe/g dry weight, (SD ± 8.57; range 0.8–47.3) and mean serum ferritin values of 2292 ng/mL (SD ± 1461; range 146–6,320). Therefore, while the mean liver iron concentration values were similar in both the thalassemia intermedia and major groups, serum ferritin levels were statistically different, with serum ferritin being significantly lower ($p$=0.003) in the thalassemia intermedia group (Figure 1). Therefore, as demonstrated in previous studies, evaluation of serum ferritin levels appears to underestimate the extent of iron overload in the thalassemia intermedia population.[3,8] It has been suggested that, in transfused patients, iron is preferentially distributed to the reticuloendothelial system and that ferritin synthesis and release is responsible for higher serum ferritin levels.[8] In contrast, in non-transfused patients, iron accumulated as a result of hyperabsorption is accumulated in hepatocytes and, therefore, lower serum ferritin levels are seen.[8] Due to limited resources, assessment of liver iron concentration is often not possible in our region. Where serum ferritin levels provide the only available indication of iron levels, we would suggest that levels below 1,000 ng/mL should not be used as a negative predictor of significant iron overload in patients with thalassemia intermedia.

In conclusion, the data presented confirm that serum ferritin levels do not accurately reflect the level of iron overload in patients with thalassemia intermedia. These observations have important implications for patient management, as assessment of serum ferritin alone may result in a delay in initiating chelation therapy and may, therefore, prolong patient exposure to high iron levels and the associated morbidity and mortality risks. Unlike thalassemia major, in which transfusion history can be a useful indicator as to whether iron chelation therapy is required, patients with thalassemia intermedia will require accurate assessment of body iron levels in order to guide therapy. As assessment of serum ferritin is evidently inappropriate in these patients, disease-specific recommendations for the management of patients with thalassemia intermedia should include direct assessment of liver iron concentration by biopsy or non-invasive imaging methods.

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## Pregnancy outcome in patients with β-thalassemia intermedia at two tertiary care centers, in Beirut and Milan

β-thalassemia intermedia (TI) patients can present with a severe clinical disease at 2-6 years of age or remain asymptomatic until adult life. They suffer from mild anemia (hemoglobin (Hb) between 7-10 g/dL), and are usually transfusion independent.[1] Pregnancy in these women, whether spontaneous or through assisted reproductive technology, represents a challenge for the treating physician. The literature is limited by the scarcity of studies about TI and pregnancy. We report on the pregnancy outcome of TI women in two tertiary care centers, the Chronic Care Center, Hazmieh, Lebanon and the Hereditary Anemia Center, Milan, Italy over a 15-year period.

During pregnancy, patients in both centers have regular antenatal visits where Hb levels are assessed every two weeks, serum ferritin every four weeks, and ultrasonographic evaluation of fetal growth every four weeks starting at 24–26 weeks. In Italy, transfusions are administered for Hb<10 g/dL whereas in Lebanon, transfusion is reserved for symptomatic patients or those with fetal growth restriction (IUGR). Maternal medical records were reviewed for several demographic and clinical variables. Women were contacted by phone for any missing information.

A total of 44 TI women who had 83 pregnancies, all spontaneous, 30 from Lebanon and 53 from Italy, were identified (Table 1). These pregnancies resulted in 17 abortions (20.5%), 64 live-births (77.1%) and 2 intrauterine fetal deaths at 26 and 36 weeks' gestation. The mean gestational age (GA) at delivery was 36.5±3.1 weeks and birthweight was 2551±621 grams. In pregnancies progressing >20 weeks' gestation (n=66), pre-term delivery and IUGR were noted in 31.8% and 24.2% respectively (Table 2). In those complicated by IUGR, cesarean delivery (CS) rate was 87.5% at GA=5.7±3.4 weeks and birthweight = 2067±530 grams. Two women (Italy) developed severe alloimmune hemolytic anemia. One progressed to cardiac failure at 35 weeks' gestation and had CS. The other underwent CS for IUGR and non-reassuring fetal heart monitoring and is scheduled for a splenectomy postpartum. Worsening alloimmune anemia also developed in 2 women in Lebanon who required splenectomy within eight weeks postpartum. Transfusion was required in 35/44 women during pregnancy (79.5%), with 27.3% requiring transfusion during pregnancy for the first time. The lowest mean Hb level was 6.7±2.0 *vs.* 8.3±1.2 g/dL in Lebanon and Italy, $p<0.001$. The average ferritin level before pregnancy was 885.2±658.9 *vs.* 1232.8±902.9 after pregnancy. CS was performed in 48 pregnancies (72.7%), the indications being elective (41.7%), repeat (31.2%) and obstetrical (27.1%). Pregnancy outcome was similar between Lebanon and Italy with the exception of a significantly higher rate of live-births in Italy (Table 2).

To the best of our knowledge, this is the largest study of pregnancy in TI women. Our results show that these pregnancies are associated with a 20.5% incidence of spontaneous abortion, 31.8% pre-term delivery, 24.2% IUGR, and 72.7% CS. This confirms the findings of a previous publication by our team that reported on 9 pregnancies, all of which were included in the current study.[2]

The chronic anemia due to thalassemia in addition to the physiological anemia in pregnancy (secondary to

**Table 1.** Patient demographics.

| | *Lebanon (n=11)* | *Italy (n=33)* | *Total (n=44)* | *p value* |
|---|---|---|---|---|
| Age at diagnosis (years) | 9.5±7.4 | 9.1±10.3 | 9.2±9.7 | 0.906 |
| Age at first transfusion (years) | 14.1±10.3 | 13.8±12.6 | 13.9±11.8 | 0.944 |
| Splenectomized | 10 (90.9) | 24 (72.7) | 34 (77.3) | 0.408 |
| Age at splenectomy (years) | 17.7±10.3 | 16.3±10.9 | 16.7±10.6 | 0.711 |
| Transfusion requirement (irrespective of pregnancy) | | | | |
| Frequently | 2 (18.2) | 12 (36.4) | 14 (31.8) | 0.456 |
| Occasionally | 8 (72.7) | 17 (51.5) | 25 (56.8) | 0.301 |
| Never | 1 (9.1) | 3 (9.1) | 4 (9.1) | 1.000 |
| Received chelation | 3 (27.3) | 25 (75.8) | 28 (63.6) | 0.009 |

*Data presented as n (%) or mean ± standard deviation. Frequently transfused = those requiring at least four transfusions/year. Occasionally transfused = those transfused in a lifetime under certain conditions such as surgery of pregnancy.*

**Table 2.** Pregnancy outcome.

| *Pregnancies* | *Lebanon (n=30)* | *Italy (n=53)* | *Total (n=83)* | *p value* |
|---|---|---|---|---|
| Abortions | 9 (30.0) | 8 (15.1) | 17 (20.5) | 0.182 |
| Live births | 19 (63.3) | 45 (84.9) | 64 (77.1) | 0.048 |
| Intrauterine fetal death[a] | 2 (6.7) | 0 | 2 (2.4) | 0.128 |
| Pre-term delivery[ab] | 8 (38.1) | 13 (28.9) | 21 (31.8) | 0.642 |
| Cesarean delivery[a] | 12 (57.1) | 36 (80.0) | 48 (72.7) | 0.100 |
| Intrauterine growth restriction[ac] | 6 (28.6) | 10 (22.2) | 16 (24.2) | 0.801 |
| Thrombotic events | 2 (6.7) | 4 (7.5) | 6 (7.2) | 1.000 |
| DVT antepartum | 1 (3.3) | 2 (3.8) | 3 (3.6) | |
| DVT in pregnancy and postpartum | 1 (3.3) | 1 (1.9) | 2 (2.4) | |
| Placental thrombosis | 0 | 1 (1.9) | 1 (1.2) | |

*Data presented as n (%). [a]After excluding abortions; DVT= deep vein thrombosis [b]defined as delivery at <37 weeks of gestation. [c]defined as <10[th] percentile for gestational age.*