β-Thalassemia intermedia: morbidity uncovered

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Chapter 2

Ineffective Erythropoiesis

Levels Of Growth Differentiation Factor-15 Are High And Correlate With Clinical Severity In Transfusion-independent Patients With β Thalassemia Intermedia

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Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent patients with β thalassemia intermedia

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ABSTRACT

Transfusion-independent patients with β thalassemia intermedia (TI) experience a variety of clinical complications attributed to the underlying ineffective erythropoiesis and subsequent anemia, hemolysis, and iron overload. Growth differentiation factor-15 (GDF-15) was recently investigated as a marker of ineffective erythropoiesis in several anemias. In this work, we evaluated GDF-15 levels in 55 patients with TI. The mean GDF-15 level was 25,197.8 ± 16,208.9 pg/ml which is lower than values reported for patients with thalassemia major, yet considerably higher than those reported in patients with other congenital and acquired anemias. GDF-15 levels were significantly higher in splenectomized compared to non-splenectomized patients and correlated with anemia, markers of iron overload, and a pre-defined clinical severity score. Further studies are needed to determine the practical utility of GDF-15 measurement and its potential to reflect the severity of the clinical course in TI patients.

Introduction

A diversity of phenotypes exists within the β thalassemia syndromes. Patients with β thalassemia intermedia (TI) are those who usually present to medical attention beyond the age of two years, and maintain hemoglobin values between 70 and 90 g/l without the need for a regular transfusional regimen [1]. However, it is now established that the diagnosis of TI carries higher morbidity than previously recognized, with clinical complications involving most organ systems and a disease severity that worsens with advancing age [2–4]. Ineffective erythropoiesis is the hallmark of disease process in TI, leading to anemia, hemolysis, and iron overload due to intestinal hyperabsorption [5,6]. Measurement of growth differentiation factor-15 (GDF-15), a member of the transforming growth factor-β superfamily, is thought to predict ineffective or apoptotic erythropoiesis [7]. GDF-15 levels were found to be significantly elevated in patients with β thalassemia major (TM). Furthermore, sera from those patients suppressed expression of the iron regulatory protein hepcidin, with a subsequent reversal of suppression when GDF-15 was depleted [8]. Data on GDF-15 levels in patient with transfusion-independent TI are lacking. Moreover, correlation between GDF-15 level, as a marker of ineffective erythropoiesis, and clinical severity in thalassemia patients was never explored.

Materials and methods

This was a cross-sectional study of all TI patients treated at two centers in Milan, Italy and Beirut, Lebanon. All patients were diagnosed with TI based on previously described criteria [2]. After excluding all patients with any history of transfusion, iron chelation, or fetal hemoglobin induction therapy, 55 patients were available for analysis. The study received institutional review board approval and written informed consent was provided by all patients. Patient charts were reviewed to retrieve data on demographics (age and gender), splenectomy status, and clinical complications known to be common in patients with TI [2] defined according to criteria described in the OPTIMAL CARE study [4]. None of the patients had hemoglobin S, C, E/β or δβ thalassemia. Moreover, none of the patients had co-inheritance of α thalassemia [α^+ ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) or α^0 ($-^{Med}$ and $-^{SEA}$)] or determinants associated with increased γ chain production [*Xmn*-I +/+ genotype at position −158 of *HβG2*].

Blood samples were obtained for assessment of hemoglobin and serum ferritin levels. For LIC, direct determination of iron burden was performed using R2 MRI in Beirut and R2* MRI in Milan using established methodologies, calibrated to mg/g of iron by dry weight (dw) in fresh liver biopsy specimens [9,10]. LIC measurements using R2* MRI are unbiased with respect to those using R2 MRI [11].

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Serum GDF-15 was evaluated using DuoSet Sandwich ELISA Kit (R&D Systems, Minneapolis, MN). Briefly, serum samples were centrifuged to remove residual cells; 96-well plates were coated with 2 μg/ml monoclonal mouse anti-human GDF-15 capture antibody and blocked with 1% bovine serum albumin in phosphate-buffered saline. After incubation with serum, the wells were washed and bound GDF-15 was detected using a biotinylated goat anti-human GDF-15 antibody. Recombinant human GDF-15 protein was used to generate a standard curve.

Patients were also assigned a clinical severity score as described in Table 1, inspired by recently published literature recently reviewed [2] as well as from clinical experience. Patients were further classified as having mild (severity score ≤5), moderate (severity score 6–10), or severe TI (severity score >10) (Table 1).

Statistical analysis

Descriptive statistics are expressed as means ± (standard deviation, SD), medians, or percentages. Bivariate correlations between GDF-15 levels and study parameters were evaluated using the independent samples *t*-test for categorical variables and the Pearson's correlation coefficient (r) for continuous variables. To determine the best GDF-15 cut-offs for discriminating patients with mild, moderate, or severe TI; the maximum sum of sensitivity and specificity was calculated from receiver-operating characteristic (ROC) curve analysis. All *P*-values are two sided with the level of significance set at <0.05.

Results and discussion

The mean age of patients was 30.7 ± 15 years (range, 8–67 years) with 25 patients (45.5%) being males. Thirty-five patients (63.3%) were splenectomized. The mean hemoglobin level was 87 ± 17 g/l (range, 51–131 g/l), while the mean serum ferritin level was 763.6 ± 501.5 μg/l (range, 18–1772 μg/l) and the mean LIC was 7.2 ± 6.6 mg Fe/g dw (range, 0.6–32 mg Fe/g dw). The mean GDF-15 level was 25,197.8 ± 16,208.9 pg/ml (range, 1126–58,262 pg/ml). GDF-15 levels increased with age (r = 0.23, $P = 0.043$), but were comparable in males and females. Mean GDF-15 levels were significantly higher in splenectomized compared to non-splenectomized patients (29,266.5 ± 15,914.8 vs. 18,077.6 ± 14,477 pg/ml, $P = 0.012$). GDF-15 levels also correlated positively with serum ferritin levels (r = 0.36, $P = 0.007$) and LIC (r = 0.63, $P = 0.014$), yet correlated negatively with hemoglobin level (r = −0.312, $P = 0.048$).

The median severity score was 7 (range, 1–15). There was a strong positive correlation between GDF-15 levels and the severity score (r = 0.830, $P < 0.001$, Fig. 1A). On ROC curve analysis, a GDF-15 cut-off of 16,000 pg/ml differentiated patients with mild from moderate TI with an area under the curve (AUC) of 0.864 ± 0.067 ($P < 0.001$), a specificity of 88.9%, and a sensitivity of 90%. Moreover, a GDF-15 cut-off of 32,000 pg/ml differentiated patients with moderate from severe TI with an AUC of 0.924 ± 0.042 ($P < 0.001$), a specificity of 85%, and a sensitivity of 88.2%.

This is the first report on GDF-15 levels in transfusion-independent patients with TI. Despite being lower than patients with TM, levels of

Table 1
Clinical severity score for patients with transfusion-independent β thalassemia intermedia.

Factor	Score
Age (years)	
≤10	0
11–20	1
21–32	2
>32	3
Splenectomy	
No	0
Yes	1
Hemoglobin level (g/dl)	
≥9	0
<9	1
LIC (mg Fe/g dry weight)	
<3	0
3 to <7	1
7 to <15	2
≥15	3
Clinical complications	
Extramedullary hematopoiesis	1
Leg ulcers	1
Thrombosis	1
Pulmonary hypertension	1
Heart failure	1
Abnormal liver function	1
Diabetes mellitus	1
Hypothyroidism	1
Osteoporosis	1
Hypogonadism	1
Minimum score	0
Maximum score	18
Mild TI	≤5
Moderate TI	6 to 9
Severe TI	≥10

LIC, liver iron concentration; TI = β thalassemia intermedia.

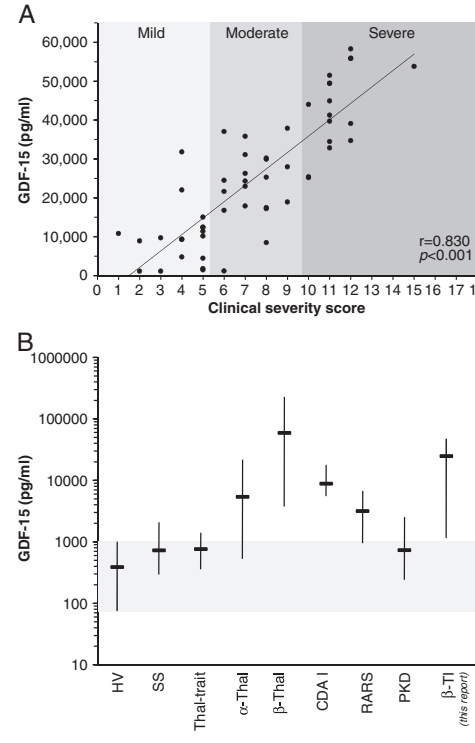

Fig. 1. A) Correlation between growth differentiation factor-15 (GDF-15) levels and clinical severity score in β thalassemia intermedia. B) GDF-15 levels from healthy volunteers (HV; n = 37), sickle cell anemia (SS; n = 13), thalassemia trait (Thal-trait; n = 12), α thalassemia (α-Thal; n = 20), β thalassemia (β-Thal; n = 40), congenital dyserythropoietic anemia type I (CDA I; n = 17), refractory anemia with ring sideroblasts (RARS, n = 20), pyruvate kinase deficiency (PKD, n = 22) [7] and transfusion-independent β thalassemia intermedia (β-TI; n = 55) (this report). Bars show the range and mean (dash) of GDF-15 concentrations.

GDF-15 in patients with TI are considerably higher than those reported in patients with other congenital and acquired anemias (Fig. 1B) [7]. Since GDF-15 has been regarded as a marker of ineffective erythropoiesis, our findings also confirm the substantial role of ineffective erythropoiesis in the pathophysiology and clinical severity of TI. Ineffective erythropoiesis, and the subsequent chronic anemia and hypoxia, lead to hepcidin suppression, increased iron absorption from the gut, and increased release of recycled iron from the reticuloendothelial system. This results in depletion of macrophage iron, relatively low levels of serum ferritin, and preferential portal and hepatocyte iron storage. This in turn leads to considerable hepatic iron overload (suggested by a positive correlation between GDF-15 levels and LIC in this report) and release into the circulation of toxic iron species like non-transferrin-bound iron, which can lead to target-organ damage [5,12–14]. Ineffective erythropoiesis is also directly related to the occurrence of osteoporosis in thalassemia [15]. Moreover, ineffective erythropoiesis results in the secondary release into the circulation of damaged red blood cells with thrombogenic potential that reflect in a high rate of thromboembolic events in TI [4,16,17], as well as an increased release of placenta growth factor, endothelin-1, which may contribute to pulmonary hypertension [4,18].

Further studies are needed to confirm the association between GDF-15 levels and erythropoietic drive in TI, since its measurement could potentially reflect the severity of ineffective erythropoiesis and subsequent clinical picture. This could allow better understanding of the phenotype heterogeneity in TI and the relative contribution of the various mechanisms implicated in the disease process.

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