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Evaluation of the levels of the markers of ineffective erythropoiesis (transforming growth factor-beta, growth differentiation factor 15 and erythropoietin) in patient with ß-thalassemia syndrome and its correlation to clinical and hematological parameters

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Abstract:

BACKGROUND: Anemia of β thalassemia results from a combination of ineffective erythropoiesis and hemolysis. This stimulates erythropoietin (EPO) production, which causes expansion of the bone marrow and may lead to serious deformities of the skull and long bones. Ineffective erythropoiesis also induces the release of growth differentiation factor 15 (GDF-15) and transforming growth factor-beta (TGF-β) which have been identified as regulators of hepcidin expression.

OBJECTIVE: The objective is to evaluate the level of TGF-β, GDF-15, and EPO in patients with thalassemia syndrome.

PATIENTS, MATERIALS AND METHODS: Patient samples were collected from Thalassemia Center of Ibn Al-Baladi Hospital. This study included 35 patients with thalassemia, 18 patients with beta-thalassemia major and 17 patients with beta-thalassemia intermedia. The age of studied group was 3–17 years. Twenty control healthy subjects were included for comparison who were age- and sex-matched with the patients group. Gel tube was used for collection of serum for enzyme-linked immunosorbent assay test for GDF-15, TGF-β, and EPO).

RESULTS: There was a highly significant difference in GDF-15 and EPO levels among studied groups ($P < 0.001$). In addition, there was no significant difference in TGF-β level among studied groups ($P > 0.05$). TGF-β, GDF-15, and EPO were not significantly correlated to splenomegaly, hepatosplenomegaly, and frequency of blood transfusion duration in patients with beta-thalassemia major ($P > 0.05$), while TGF-β and EPO were significantly correlated to splenomegaly, hepatosplenomegaly in patients with beta-thalassemia intermedia but GDF-15 was not significantly correlated. In patients with beta-thalassemia major, EPO was negatively correlated to hemoglobin, packed cell volume, mean corpuscular volume, and red blood cells (RBC) count whereas GDF-15 significantly correlated to lymphocyte and neutrophil counts. TGF-β was significantly correlated to platelet count. In patients with beta-thalassemia intermedia, EPO and GDF-15 were not correlated to any hematological parameters whereas TGF-β was significantly correlated to RBC counts.

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CONCLUSION: Marker of erythropoiesis GDF-15, EPO was highly expressed in patient with beta-thalassemia major and beta-thalassemia intermedia as compared to the control group and this can be used as a future therapeutic goal for the suppression of ineffective erythropoiesis.

Keywords:

Erythropoietin, growth differentiation factor 15, transforming growth factor-beta, thalassemia syndrome

Introduction

Thalassemias are groups of hereditary blood disorders characterized by anomalies in the synthesis of α-or β-chains of hemoglobin (Hb) called α-thalassemia and β-thalassemia, respectively. Together with sickle cell anemia, the thalassemias are the most common inherited disorders of Hb.[1]

Beta thalassemia is the result of a deficient or absent synthesis of beta-globin chains, leading to relative excess in the alpha chains. Beta globin synthesis is controlled by one gene on each chromosome.[2]

β-Thalassemias are characterized by ineffective erythropoiesis due to the imbalance of globin chain production, resulting in increased apoptosis during erythroblast maturation.[3]

Although hepcidin concentration is increased in conditions of primary iron overload, diseases of concurrent anemia and iron overload (i.e., increased and ineffective erythropoiesis) are associated with relatively suppressed hepcidin levels.[4,5]

The combination of ineffective erythropoiesis and hemolysis leads to anemia, hypoxia, and increased erythropoietin (EPO) production.[6,7]

In β-thalassemias, ineffective erythropoiesis cause the release of growth differentiation factor 15 (GDF15), twisted gastrulation protein homolog 1, which inhibits hepcidin.[6] The expansion of the erythroid compartment leads to the over expression of GDF15, which inhibits the expression of hepcidin, ultimately leading to iron overload.[8]

Transforming growth factor beta (TGF-β) super family members have been identified as regulators of hepcidin expression, It regulates late-stage erythropoiesis-The

TGF-β superfamily regulates Smad2/3 signaling.[9]

Patients, Materials and Methods

Thirty-five patient samples were collected from thalassemia center of Ibn Al-Baladi hospital. The patients randomly selected regarding sex. The age was between 3 and 17 years.

Twenty control samples were collected from Al-kadhmiya pediatric hospital who were age- and sex-matched with the patients' group.

This study is included 35 patients with thalassemia, 18 patients with thalassemia major and 17 patients with thalassemia intermedia and 20 control leftover samples, who were age- and sex-matched with the patients group.

This study was approved by the review ethical committee of the department of pathology and forensic medicine of Al-Nahrain University/College of Medicine. All Patients and healthy controls agreed to participate in this study and they signed written informed consent All clinical information related to splenomegaly hepatosplenomegaly were taken from patients file at the time of investigation before blood transfusion, some investigation like serum ferritin was taken either directly from the patients or the nearest one that found in the patients file.

Each serum sample after collection was put in a deep freeze-20c and after the collection of all samples; the investigation was done to all samples at the same time.

Limitation of the study

Newly transfused patients who take blood in a period of <2 weeks.

Gel tube was used for collection of serum for enzyme-linked immunosorbent assay (ELISA) test (GDF-15, TGFB and EPO).

ELISA test was done for:

GDF15 by Elabscience USA

This ELISA kit uses the Sandwich-ELISA principle. The ELISA plate has been precoated with an antibody specific to Human GDF15.

Standards or samples are added to the plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human GDF15 and Avidin-horseradish peroxidase (HRP) conjugate are added well and incubated.

Free components are washed away. The substrate solution is added to each well.

Only those wells that contain human GDF-15, biotinylated detection antibody, and Avidin-HRP conjugate will appear blue. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow.

Transforming growth factor beta by DRG transforming growth factor-β1 enzyme-linked immunosorbent assay the USA

This ELISA kit uses the Sandwich-ELISA principle also.

Erythropoietin by DRG erythropoietin enzyme-linked immunosorbent assay USA

The DRG EPO Immunoassay is a two-site ELISA for the measurement of EPO. It utilizes two different mouse monoclonal antibodies to human EPO specific for well-defined regions on the EPO molecule. One mouse monoclonal antibody to human EPO is biotinylated and the other mouse monoclonal antibody to human EPO is labeled with HRP for detection.

Results

There was a highly significant difference in GDF-15 and EPO levels among studied groups ($P < 0.001$); the level being highest in beta-thalassemia major, followed by beta-thalassemia intermedia and then by the control group. In addition, there was no significant difference in TGF-β level among studied groups ($P > 0.05$) as shown in Table 1.

TGF-β, GDF-15, and EPO were not significantly correlated to splenomegaly, hepatosplenomegaly, and frequency of blood transfusion duration in patients with beta-thalassemia major ($P > 0.05$). While TGF-β and EPO were significantly correlated to splenomegaly, hepatosplenomegaly in patients with beta-thalassemia intermedia but GDF-15 was not significantly correlated as shown in Tables 2 and 3.

In patients with beta-thalassemia major, EPO was negatively correlated to Hb, packed cell volume (PCV), mean corpuscular volume (MCV), and red blood cells (RBC) count whereas GDF-15 was significantly correlated to lymphocyte and neutrophil counts. TGF-β was significantly correlated to platelet count as shown in Table 4.

In patients with beta-thalassemia intermedia, EPO and GDF-15 were not correlated to any hematological parameters in patients with beta-thalassemia intermedia, whereas TGF-β was significantly correlated to RBC counts as shown in Table 5.

Discussion

Ineffective erythropoiesis, due to excess production of free alpha (α)-globin chains, is the hallmark of beta (β)-thalassemia. Ineffective erythropoiesis results in severe anemia and leads to a number of compensatory mechanisms responsible for the clinical severity of β-thalassemia such as erythroid marrow expansion, extramedullary hematopoiesis, splenomegaly, and increased gastrointestinal iron absorption. The increase in plasma volume caused by marrow expansion and splenomegaly exacerbate anemia and increase transfusion requirements.[6]

The anemia and hypoxia associated with β-thalassemia lead to an increase in serum levels of EPO as the body attempts to compensate for the reduced oxygen-carrying capacity of the blood. However, the thalassemic erythroid marrow is unable to respond adequately to EPO, resulting in erythroid hyperplasia and massive expansion of the erythroid marrow without increase in the number of mature erythrocytes in the peripheral blood.[8]

Table 1: Comparison of transforming growth factor beta, growth differentiating factor-15 and erythropoietin among patients with thalassemia major, thalassemia intermedia and control subjects

Characteristic	Thalassemia major (*n*=18)	Thalassemia intermedia (*n*=17)	Control (*n*=20)	*P*
TGF-β1				
Median (IQR)	145.76 (139.61)	188.65 (65.61)	137.32 (164.15)	0.276 K (NS)
	A	A	A	
Range	53.47–360.04	61.94–480.20	48.03–266.95	
GDF-15				
Median (IQR)	1158.40 (240.33)	506.35 (549.22)	198.94 (158.96)	<0.001 K (HS)
	A	B	C	
Range	1018.11–1383.97	54.12–1150.73	65.42–430.24	
EPO				
Median (IQR)	49.46 (96.44)	16.54 (12.83)	13.26 (12.68)	<0.001 K (HS)
	A	B	B	
Range	15.02–243.44	5.67–41.72	6.25–104.50	

Capital letters (A, B and C) were used to indicate the level of significance following post hoc Dunn's test so that similar letters indicate no significant difference whereas, different letters indicate significant difference. *n*=number of cases, IQR=Interquartile range, K=Kruskal–Wallis test, HS=Highly significant at $P \leq 0.01$, NS=Not significant at $P>0.05$, S=Significant at $P \leq 0.05$, GDF-15=Growth differentiating factor 15, TGF-β1=Transforming growth factor beta1, EPO=Erythropoietin

Table 2: Correlations of transforming growth factor beta, growth differentiating factor-15 and erythropoietin to splenectomy, hepatosplenomegaly and frequency of blood transfusion duration in patients with beta thalassemia major

Characteristic	TGFB1		GDF15		EPO	
	r	*P*	*r*	*P*	*r*	*P*
Splenomegaly	0.085	0.746 (NS)	0.425	0.089 (NS)	0.227	0.382 (NS)
Hepatosplenomegaly	−0.283	0.271 (NS)	−0.226	0.382 (NS)	−0.085	0.746 (NS)
Frequency of blood transfusion	0.043	0.871 (NS)	−0.189	0.468 (NS)	−0.451	0.069 (NS)

r=Correlation coefficient, NS=Not significant at *P*>0.05, S=Significant at *P*≤0.05, GDF-15=Growth differentiating factor 15, TGF-β1=Transforming growth factor beta, EPO=Erythropoietin

Table 3: Correlations of transforming growth factor beta, growth differentiating factor -15 and erythropoietin to splenectomy, hepatosplenomegaly and frequency of blood transfusion duration in patients with beta thalassemia intermedia

Characteristic	TGFB1		GDF15		EPO	
	r	*P*	*r*	*P*	*r*	*P*
Splenomegaly	0.472	0.048 (S)	0.121	0.633 (NS)	0.527	0.024 (S)
Hepatosplenomegaly	−0.472	0.048 (S)	−0.121	0.633 (NS)	−0.527	0.024 (S)
Frequency of blood transfusion	0.339	0.169 (NS)	0.323	0.191 (NS)	−0.185	0.462 (NS)

r=correlation coefficient, NS=Not significant at *P*>0.05, S=Significant at *P*≤0.05, GDF-15=Growth differentiating factor 15, TGF-β1=Transforming growth factor beta, EPO=Erythropoietin

Ineffective erythropoiesis leads to increased EPO, and GDF15 suggesting that, despite RBC transfusions, expanded erythropoiesis is likely to present but ineffective.[8]

The TGF-β family negatively regulates the erythrocyte differentiation and maturation, whilst EPO is the regulator of early-stage erythropoiesis. TGF-β signaling inhibits erythroid differentiation by inducing apoptosis and cell cycle arrest in erythroblasts.[10]

β-thalassemia patients with the same gene mutation demonstrate remarkable diversity in hematological and clinical symptoms. Several factors are involved in the disease severity of β-thalassemia such as environmental or genetic modifiers.[11] For example, the hereditary persistence of fetal Hb and simultaneous α-globin chain mutations can change the globin chain balance and reduce the destructive effect of free α-globin.[12]

- Regarding the comparison of TGF-β, GDF-15 and EPO among our patients with thalassemia major, thalassemia intermedia and control subjects. There were a highly significant difference in GDF-15 level and EPO level among studied groups, the level being highest in beta thalassemia major, followed by beta thalassemia intermedia and then by control group. The combination of ineffective erythropoiesis and peripheral hemolysis leads to anemia, hypoxia and this leads to increased EPO production. EPO is responsible for stimulating and regulating the erythropoiesis rate. The expansion of the erythroid compartment leads to the over expression of GDF15, which inhibits the expression of hepcidin, ultimately leading to iron overload.[13] This were in agreements with other studies Rivella.[13,14]
- Regarding the TGF-β level in this study showed increment in 56% of thalassemia major patients and 88% of thalassemia intermedia patients but median show no difference between the compared groups. This was comparable with other results Tanno *et al*.[8] who found that there is increment in the level of TGF-β but also not reach the level of significance
- This result was disagree with other studies Al-Hindy *et al*.[15,16] whom found TGF-β was significantly increased in thalassemia major and thalassemia intermedia compared to control group. This difference may be related to blood transfusion or sample size or other modifiers
- Our study showed no significant correlation between TGF-β, to splenomegaly, hepatosplenomegaly and frequency of blood transfusion duration in patients with beta thalassemia major ($P > 0.05$), while in patients with beta-thalassemia intermedia TGF-β was significantly correlated to splenomegaly and hepatosplenomegaly
- The GDF15 showed no significant correlation to splenomegaly, hepatosplenomegaly and frequency of blood transfusion duration in patients with beta thalassemia major and beta-thalassemia inermedia. The *P* value was >0.05. This disagree with other studies Musallam *et al*.[2,17] whom found GDF-15 correlate with anemia, iron overload, and clinical severity
- EPO showed no significant correlation to splenomegaly, hepatosplenomegaly and frequency of blood transfusion duration in patients with beta thalassemia major. Although the *P* value of the correlation between EPO and frequency of blood transfusion in thalassemia major was (0.06) which was close to the level of significance.
- EPO was significantly correlated to splenomegaly, hepatosplenomegaly in patients with beta thalassemia intermedia
- Regarding the correlation of markers of ineffective erythropoiesis and hematological parameters. In our study, EPO was negatively correlated to Hb, PCV, MCV and RBC count in patients with beta thalassemia Major, Comparable results were found Tanno *et al*.,[8,10] In beta thalassemia, Epo was dramatically

Table 4: Correlations of transforming growth factor beta, growth differentiating factor-15 and erythropoietin to hematological parameters in patients with beta thalassemia major

Characteristic	TGFB1		GDF15		EPO	
	r	*P*	*r*	*P*	*r*	*P*
Hb	0.139	0.595 (NS)	0.091	0.729 (NS)	−0.746	0.001 (HS)
PCV	0.209	0.421 (NS)	0.082	0.753 (NS)	−0.590	0.013 (S)
MCV	0.164	0.529 (NS)	0.152	0.561 (NS)	−0.519	0.033 (S)
MCH	−0.095	0.718 (NS)	0.275	0.285 (NS)	−0.415	0.098 (NS)
MCHC	0.021	0.937 (NS)	−0.128	0.626 (NS)	0.179	0.493 (NS)
RBC	0.188	0.470 (NS)	0.067	0.798 (NS)	−0.725	0.001 (HS)
Ferritin	0.269	0.297 (NS)	0.222	0.392 (NS)	−0.031	0.907 (NS)
Platelet	−0.492	0.045 (S)	−0.141	0.589 (NS)	−0.040	0.877 (NS)
WBC	−0.190	0.465 (NS)	−0.139	0.596 (NS)	−0.233	0.368 (NS)
Lymphocyte	0.077	0.768 (NS)	0.508	0.037 (S)	0.004	0.989 (NS)
Neutrophil	−0.087	0.741 (NS)	−0.532	0.028 (S)	−0.089	0.734 (NS)
Monocytes	−0.075	0.775 (NS)	0.194	0.456 (NS)	0.013	0.960 (NS)
Eosinophils	0.131	0.617 (NS)	−0.237	0.359 (NS)	0.180	0.490 (NS)
Basophils	0.153	0.557 (NS)	0.153	0.557 (NS)	0.204	0.432 (NS)

r=Correlation coefficient, NS=Not significant at *P*>0.05, S=Significant at *P*≤0.05, GDF-15=Growth differentiating factor 15, TGF-β1=Transforming growth factor beta, EPO=Erythropoietin, Hb=Hemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobincocentration, RBC=Red blood cells

Table 5: Correlations of transforming growth factor beta, growth differentiating factor-15 and erythropoietin to hematological parameters in patients with beta thalassemia Intermedia

Characteristic	TGFB1		GDF15		EPO	
	r	*P*	*r*	*P*	*r*	*P*
Hb	0.329	0.182 (NS)	0.139	0.583 (NS)	−0.114	0.653 (NS)
PCV	0.314	0.204 (NS)	0.110	0.665 (NS)	−0.136	0.590 (NS)
MCV	0.183	0.468 (NS)	0.007	0.977 (NS)	−0.142	0.575 (NS)
MCH	0.168	0.506 (NS)	−0.132	0.600 (NS)	0.014	0.956 (NS)
MCHC	0.062	0.806 (NS)	0.107	0.673 (NS)	0.266	0.287 (NS)
RBC	0.518	0.028 (S)	0.437	0.069 (NS)	−0.116	0.646 (NS)
Ferritin	0.068	0.788 (NS)	0.346	0.159 (NS)	−0.053	0.835 (NS)
Platelet	0.040	0.874 (NS)	−0.183	0.468 (NS)	−0.045	0.858 (NS)
WBC	0.084	0.742 (NS)	0.199	0.428 (NS)	0.205	0.413 (NS)
Lymphocyte	−0.168	0.505 (NS)	−0.092	0.715 (NS)	−0.292	0.240 (NS)
Neutrophil	0.195	0.437 (NS)	0.081	0.750 (NS)	0.330	0.182 (NS)
Monocytes	−0.302	0.223 (NS)	−0.227	0.364 (NS)	−0.424	0.080 (NS)
Eosinophils	−0.076	0.764 (NS)	−0.213	0.397 (NS)	−0.035	0.889 (NS)
Basophils	*	*	*	*	*	*

*Basophil is constant in patients with beta thalassemia major. *r*=Correlation coefficient, NS=Not significant at *P*>0.05, S=Significant at *P*≤0.05, GDF-15=Growth differentiating factor 15, TGF-β1=Transforming growth factor beta, EPO=Erythropoietin, Hb=Hemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobincocentration, RBC=Red blood cells, WBC=White blood cells

increased in response to anemia and hypoxia.[10] Epo showed a negative correlation with Hb level[8]

- EPO was not correlated to any hematological parameters in patients with beta-thalassemia intermedia
- The GDF-15 was significantly correlated to lymphocyte count and neutrophil count in patients with beta thalassemia major. no similar result was found, whereas GDF-15 was not correlated to any hematological parameters in patients with beta thalassemia intermedia
- The TGF-β was significantly correlated to platelet count in patients with thalassemia major, whereas TGF-β was significantly correlated to RBC count in patients with beta thalassemia intermedia.

Conclusion

Marker of erythropoiesis GDF-15 and Erythropoitin were highly expressed in patients with beta thalassemia major and beta thalassemia intermedia as compared to control group.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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