Original Article

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Quick Response Code:

Website:
www.jahjournal.org
DOI:
10.4103/joah.joah_98_22

Evaluation of the Serum Level of Vascular Endothelial Growth Factor in Patients with Beta-Thalassemia Major and Sickle/Beta-Thalassemia, and its Correlation with Clinical and Laboratory Parameters

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Abstract:

BACKGROUND: The most prevalent inherited disorders of red blood cells are hemoglobinopathies, with thalassemia and sickle cell diseases (SCDs) being the most common. In SCD and thalassemia major, angiogenesis has been identified as a substantial contributor to vascular-mediated tissue damage. Vascular endothelial growth factor (VEGF) is the master regulator of angiogenesis. This study aims to assess the circulating level of serum VEGF in beta-thalassemia (β-thal) and sickle β-thal patients and also to explore the correlation with clinical and laboratory data.

PATIENTS, MATERIALS AND METHODS: This is a cross-sectional study conducted on 80 individuals, clinical data were gathered, complete blood count, serum ferritin, and serum VEGF tests were done.

RESULTS: Patients' age ranged from 1.5 to 17.5 years, males formed (70%). Platelet count was significantly higher in β-thal compared to sickle/β-thal (S/β-thal) patients, with a $P = 0.015$. Mean serum ferritin in patients was significantly higher in β-thal compared to S/β-thal patients, $P < 0.001$. Patients' serum VEGF levels were noticeably higher than controls with $P = 0.01$. Strong positive correlation of serum VEGF with platelet count among the patients ($r = 0.603$, $P < 0.001$). A significant positive correlation was observed between serum VEGF and the age of starting chelation therapy in thalassemic patients ($r = 0.475$, $P = 0.006$).

CONCLUSIONS: Serum VEGF levels were significantly higher in patients compared to healthy controls, and there is a significant positive correlation between serum VEGF levels and the age at which iron chelation therapy was initiated as well as between serum VEGF levels and platelet counts in patients.

Keywords:

Sickle β-thalassemia, vascular endothelial growth factor, β-thalassemia major

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Submitted: 03-Nov-2022
Revised: 07-Apr-2023
Accepted: 03-May-2023
Published: 27-Jul-2023

Introduction

The Mediterranean basin, Africa, and Asia have significant incidence rates for hemoglobinopathies, which are among the most prevalent inherited disorders worldwide. Despite being uncommon among the native Central European people.[1] They can be divided into two primary categories: structural hemoglobin variants and thalassemia syndromes. The three primary forms of thalassemia with clinical significance are α, β, and δβ. The

How to cite this article: Abdul Mohsin SA, Al-Bayaa IM, Al-Badran IM. Evaluation of the serum level of vascular endothelial growth factor in patients with beta-thalassemia major and sickle/beta-thalassemia, and its correlation with clinical and laboratory parameters. J Appl Hematol 2023;14:115-21.

four most common and clinically significant structural hemoglobin variations are hemoglobin S (HbS), HbE, HbC, and HbD.[2] The subtypes of thalassemias include β0 thalassemias, in which normal-globin subunit synthesis is completely absent, and β+ thalassemias, in which some-globin subunits with typical structural characteristics are generated but at significantly lower levels.[3] A group of inherited diseases known as sickle cell disease (SCD), which includes sickle cell anemia, sickle hemoglobin C disease (HbSC), sickle/beta-thalassemia disease (S/β-thal), and many other compound heterozygous conditions, are characterized by the predominance of hemoglobin S within erythrocytes.[4] Iron overload results after multiple blood transfusions in patients with thalassemia major and S/β-thal.[5] Serum ferritin is used to assess iron overload. Iron chelation therapy improves both survival and complication rate, while iron overload has a detrimental influence on overall survival and morbidity.[6] Intravascular hemolysis causes dysfunction of endothelium, vascular proliferation, prooxidant, and proinflammatory stress because nitric oxide (NO) is scavenged by cell-free hemoglobin, which is released into the bloodstream together with red blood cell arginase, which breaks down arginine, the precursor to NO generation.[7] Angiogenesis is crucial for healing of wound and for reestablishing blood flow to tissues following trauma or shock. it is controlled in healthy physiology by inhibitors and angiogenic growth factors like vascular endothelial growth factor (VEGF). When control is lost, too many or not enough blood vessels are created.[8] VEGF is primarily mediated pathogenic effects due to its effects on vascular permeability and neoangiogenesis.[9] This study aims to assess the circulating level of serum VEGF in β-thal major and S/β-thal patients. Also, to explore the correlation with clinical and laboratory data including history of splenectomy, frequency of blood transfusion, starting age of blood transfusion, use of iron chelating agents, serum ferritin level, and other hematological parameters.

Patients, Materials, and Methods

An analytical cross-sectional study was performed on 80 individuals divided into two groups: Group I: 60 patients with hemoglobinopathy registered at Basrah Center for Hereditary Blood Disease, they were previously diagnosed by consultant hematologists based on clinical features and laboratory findings including peripheral blood examination, sickling test and HPLC from early years of their life. Those patients are further subdivided into two subgroups; 35 patients with β-thal major (group TM) and 25 patients with S/β-thal (group SCD). Group II: 20 age- and sex-matched apparently healthy individuals by history taking, non-anemic according to the WHO definition of anemia.[10]

A consent was verbally obtained from the patient him/herself or their caregiver before enrollment in the study. Furthermore, the study was approved by the ethical committee of the Scientific Council of Pathology at the Iraqi Board for Medical Specializations and by the research ethics committee of Basrah Health Directorate.

All relevant clinical data were collected according to a questionnaire form by direct interview with the patients and/or their caregivers. Any patient with acute illness and fever, history of recent blood transfusion in the past 3 weeks, presence of other hemoglobinopathies, any solid or hematological malignancy, diabetes mellitus, rheumatoid arthritis, and other autoimmune diseases was excluded from the study.

From each participant in this study, a volume of 5 mL of peripheral venous blood sample was collected by venipuncture under aseptic technique. Two ml of blood was poured into EDTA tube and tested for complete blood count (CBC), the residual blood collected into gel tube was allowed to clot in waterbath at 37°C then centrifuged at 2000 g for 5 min to get serum, that was used to measure serum ferritin by using cobas e411 analyzer (Roche Diagnostics), then separated in small aliquots and kept in deep freezer (below-40°C) until serum VEGF assay was done by solid phase sandwich enzyme-linked immunosorbent assay (ELISA) using Human VEGF Quantikine ELISA Kit (R&D system, USA).

Statistical analysis

The statistical analysis was done using theStatistical package for social sciences version 26, IBM SPSS statistics for windows (Armonk, NY: IBM Corp). While continuous numerical data were represented as mean ± standard deviation, qualitative data were expressed as numbers and percentages. Student's *t*-test was used to compare numerical data between study groups, while the Chi-square test was used to analyze categorical variables. Correlations had been assessed using Pearson's correlation. $P < 0.05$ was statistically significant.

Results

Eighty participants with ages ranged from 1.5 to 17.5 years. A significant difference in the age between TM group (8.14 ± 3.65) years, and SCD group (12.36 ± 4.1) years, $P < 0.001$. Forty-two patients were males in the patient's group with male: female ratio = 2.3:1.

Fifty-seven out of sixty patients received blood transfusion. Different types of chelation therapy were used for different patients. There was significant difference between TM group and SCD group, in regard

to age of starting, blood transfusion and chelation therapy, with a P <0.001 and 0.046, respectively [Table 1].

The platelet count of the TM group was significantly higher compared to the SCD group, with a P = 0.015 while there were no significant differences between the two groups regarding white blood cell (WBC) and Hb (P = 0.419, 0.116, respectively). Serum ferritin level was significantly higher in TM group compared to SCD group with P < 0.001, and serum VEGF level was significantly higher in patients compared to controls with P = 0.01. However, no significant difference was observed between TM group and SCD group (P = 0.1) [Table 2].

Between the patients' serum VEGF levels and platelet counts, there was a significant positive correlation with P < 0.001. A significant positive correlation was also found when each group of patients was tested separately with P values (0.002 and < 0.001) for TM and SCD groups, respectively. While, no significant correlations were found between serum VEGF level and Hb, WBC, and serum ferritin level, it was negatively correlated with Hb [Table 3].

When patients with and without splenectomy were compared in terms of serum VEGF, a significant difference was noticed between the two groups with a P < 0.001, similarly, significant differences were found when each subgroup of patients tested separately with P values (0.006 and 0.001) among TM and SCD groups, respectively. Higher mean values were seen in splenectomized groups as described in Table 4.

Table 1: Clinical parameters of the patient's study groups

Variable	Group		*P*
	TM, *n* (%)	SCD, *n* (%)	
Frequency of blood transfusion			
Every 1 month	34/35 (97.1)	5/22 (22.7)	
Every 3 months	1/35 (2.9)	4/22 (18.2)	
Episodic	0/35 (0)	13/22 (59.1)	
Compliance to blood transfusion			
Compliant	34/35 (97.1)	22/22 (100)	
Noncompliant	1/35 (2.9)	0/22 (0)	
Age of starting blood transfusion (months), mean±SD	7.4±3.6	37.4±33	<0.001*
Chelation therapy			
Yes	32/35 (91.4)	8/22 (36.4)	
No	3/35 (8.6)	14/22 (63.6)	
Compliance to chelation therapy			
Compliant	26/32 (81.25)	6/8 (75)	
Noncompliant	6/32 (18.75)	2/8 (25)	
Age of starting chelation therapy (years), mean±SD	4.4±1.7	5.9±2.4	0.046*
Type of chelation therapy			
Deferoxamine	2/32 (6.25)	0/8 (0)	
Deferasirox	28/32 (87.5)	8/8 (100)	
Combined	2/32 (6.25)	0/8 (0)	
Splenectomy			
Yes	7/35 (20)	7/25 (28)	
No	28/35 (80)	18/25 (72)	

TM=β thalassemia major; SCD=Sickle cell disease (sickle/β thalassemia); SD=Standard deviation

Table 2: Comparison of complete blood count parameters, serum ferritin and vascular endothelial growth factor between study groups

	Group			*P*
	Patients (*n*=60)		Controls (*n*=20)	
	TM (*n*=35)	SCD (*n*=25)		
Hb (g/dL), mean±SD	8.17±1.39	8.82±1.75		0.116
WBC (×10^9/L), mean±SD	12.95±5.61	11.79±5.07		0.419
Platelet (×10^9/L), mean±SD	509.56±203.2	378.92±192.2		0.015*
Serum ferritin level (ng/mL), mean±SD (range)	2371.7±1438.2 (582.3–5886)	609±558.4 (64.6–1800)		<0.001*
VEGF level (pg/mL), mean±SD (range)	603.29±378.99 (115.71–1827.76)		374.45±142.89 (100.30–659.65)	0.01*
	671.32±378.6 (268.1–1827.76	508.04±365.84 (115.71–1630.76)		0.1

*Student's *t*-test (significant at P<0.05). TM=β thalassemia major; SCD=Sickle cell disease (sickle/β thalassemia); VEGF=Vascular endothelial growth factor; Hb=Hemoglobin; WBC=White blood cell; SD=Standard deviation

In the TM group, a significant positive correlation was noticed between serum VEGF and the age of starting chelation therapy, $P = 0.006$. Details are shown in Table 5.

There was no significant difference ($P = 0.860$) between serum VEGF level and the frequency of blood transfusion among SCD, utilizing one-way ANOVA test. While all the patients in the TM group received blood transfusion regularly [Table 6].

No significant difference was seen in the serum VEGF level across different types of chelation therapy used in the TM group ($P = 0.206$), however, all patients in the SCD group received a single type of chelation therapy (deferasirox) [Table 7].

Table 3: Correlation of serum vascular endothelial growth factor level with laboratory findings

Parameter	Pearson correlation	*P*
Hb (g/dL)	−0.044	0.740
WBC (×10^9/L)	0.22	0.088
Platelets (×10^9/L)	0.603	<0.001*
Serum ferritin level	0.228	0.08

Pearson correlation *significant at $P<0.05$. Hb=Hemoglobin; WBC=White blood cell; SD=Standard deviation

Table 4: Association between serum vascular endothelial growth factor level and splenectomy status

Serum VEGF level (pg/mL)	Splenectomy status, mean±SD		*P*
	Splenectomy	Nonsplenectomy	
All patients	944.31±444.86	499.5±289.76	<0.001*
TM (*n*=35)	1011.33±436.38	586.32±317.36	0.006*
SCD (*n*=25)	877.3±477.37	364.44±174.77	0.001*

*Significant at $P<0.05$. TM=β thalassemia major; SCD=Sickle cell disease (sickle/β thalassemia); VEGF=Vascular endothelial growth factor; SD=Standard deviation

Table 5: Correlation of serum vascular endothelial growth factor level with age of starting blood transfusion and age of starting chelation therapy

Parameter	TM		SCD	
	Pearson correlation	*P*	Pearson correlation	*P*
Age of starting blood transfusion	−0.284	0.098	0.221	0.322
Age of starting chelation therapy	0.475	0.006	−0.338	0.328

TM=β thalassemia major; SCD=Sickle cell disease (sickle/β thalassemia)

Table 6: Correlation of serum vascular endothelial growth factor level with the frequency of blood transfusion among sickle cell disease group

	Frequency of blood transfusion among SCD			*P*
	Every 1 month	Every 3 months	Episodic	
Serum VEGF level (pg/mL)	464.86±311.91	554.27±188.74	577.11±444.36	0.860

SCD=Sickle cell disease (sickle/β thalassemia); VEGF=Vascular endothelial growth factor

Discussion

β-thal syndromes and SCD are both frequent causes of inherited anemias worldwide with high prevalence in the Mediterranean region, they can lead to a broad range of complications when managed inadequately and late causing the patients a great deal of morbidity.[1,2] VEGF is a fundamental regulator of the process of angiogenesis with tissue hypoxia being an important factor stimulating its production.[11] The current study aims to investigate the role of VEGF in patients with β-thal major and S/β-thal and its correlation with clinical and laboratory parameters.

In the current study, the majority of patients were male with M: F of 2.3:1, which were closely similar to other Iraqi study by Kadhim *et al*.[12]

With respect to hematological parameters, no significant difference was seen between the 2 patient's subgroups with respect to hemoglobin level and WBC count, which is similar to a study done by Koren *et al*.[13] in 2010. While platelet count was noticed to be significantly higher in the TM group compared to the SCD group, which is similar to an Egyptian study.[14] The mean platelet count in both groups was high, which is comparable to the count in studies,[15,16] but lower means were observed in other studies,[17-19] this variation could be linked to the different number of patients who underwent splenectomy in various studies.

Almost all the patients in this study received blood transfusion except for three ones, who were S/β-thal patients with a very mild phenotype owing to high HbA levels as was documented by their original HPLC. Patients with β-thal major were subdivided into two subgroups regarding their frequency of receiving blood transfusion; every one month and every three months, with only one patient falls in the second category. Patients in the second category might have a co-inherited α thalassemia that had decreased disease severity reflected as slightly prolonged interval between each transfusion;[20] however, to clarify the cause, the patient needs a family study and genetic analysis. Similarly, patients with S/β-thal were also subclassified according to the frequency of blood transfusion, but into three categories; every one month, every 3 months, and patients who only receive blood transfusion episodically. In our study, 22.7%, 18.2%, and 59.1% of S/β-thal patients were receiving blood transfusion every 1 month, every 3 months and episodically, respectively. This variation in the need for blood transfusion can be explained by the different genotypes of S/β-thal, the inheritance of different β-thal alleles, presence or absence of Xmnl polymorphism, as well as the possible coinheritance of α thalassemia as has been observed by Mukherjee *et al*.[21]

Table 7: Correlation of serum vascular endothelial growth factor level with the type of chelation therapy

	Type of chelation therapy			*P*
	Deferoxamine	Deferasirox	Combined	
Serum VEGF level (pg/mL)	1047.93±1102.85	629±333.43	949.82±169.24	0.206

VEGF=Vascular endothelial growth factor

The age of starting blood transfusion was (7.4 ± 3.6) months and (37.4 ± 33) months in β-thal major and S/β-thal, respectively. Regarding β-thal major, this finding was comparable to a study done in Iraq[22] but a slightly lower age was documented in another study, also in Iraq by Sadullah *et al*.[23] However, the mean age was higher among patients with S/β-thal, but the age range was (5–135) months with 6 patients who received blood transfusion within the 1st year of their life, who were sickle/β-thal. The patients were found highly adherent to blood transfusion with a compliance up to 97.1% and 100% in TM and SCD groups, respectively.

Patients with TM were found to have higher serum ferritin levels compared to patients with SCD, and this finding was comparable to Voskaridou *et al*.,[24] which can be explained by the higher frequency of blood transfusion among the TM group. The mean serum ferritin was higher than in Sherief *et al.* study,[25] and lower than values obtained in other studies,[26,27] this variation in results can be attributed to the differences in the age of starting chelation therapy, types of chelation agents and variability in patients' compliance in different studies. Since the human body lacks a mechanism to eliminate extra iron, iron overload is an expected problem among patients receiving long-term blood transfusions. Chelation therapy should be started before iron buildup has reached dangerous levels to help lessen the iron burden. Compared to patients with SCD, patients with β-thal major experience an earlier onset of iron overload, iron-induced liver disease, and endocrine abnormalities.[28] This can explain the earlier age of starting chelation among TM patients compared to SCD patients in our study. Chelation therapy was initiated in all but three patients of the β-thal major group, one of them was at the age of 1.5 years and the other two patients refused chelation therapy. The mean age of starting chelation therapy was (4.4 ± 1.7) years. This was similar to age observed by other studies,[29] but it was lower in other studies.[28,30] Regarding patients with S/β-thal, only 36.4% of the transfused patients were kept on iron chelation therapy, this percentage is considerably lower than the finding obtained by Fung *et al*.[31] with the mean age of starting chelation was higher than thalassemic patients owing to the less transfusional requirement among these patients resulting in a longer time to the development of iron overload, as it was agreed by Lucania *et al*.[32] Types of chelation therapy that were used for thalassemic patients included: deferasirox, deferoxamine, and a combination of both, with 87.5% of them receiving only deferasirox, 6.25% on deferoxamine and 6.25% were receiving combination therapy. This finding was somewhat similar to an Iraqi study by Sadullah *et al*.[23] and another study in the United States.[33] Regarding patients with S/β-thal, they were all kept on deferasirox. The ease of use and free distribution of oral chelation therapy (deferasirox) by the Ministry of Health to all thalassemia institutions in Iraq are the primary explanations for the high usage of this treatment in our study. These factors also contribute to the high adherence of our patients to chelation therapy.

Serum VEGF level was significantly higher among patients compared to controls with $P = 0.01$, this is comparable to other studies,[14,30,34] while no significant difference was found between the two patients' subgroups. Tissue hypoxia, which is the primary cause of the up-regulation of VEGF, can be used to explain why patients have elevated serum levels of VEGF.[8] There was a noticeable variation in the mean VEGF between different studies,[34,35] these variations may be due to the different ELISA kit suppliers, different sample sizes, and different platelet counts among the studies. Furthermore, mean serum VEGF was noticeably higher in splenectomized than non-splenectomized patients, with $P < 0.001$. This agrees with studies done by others.[17,30,34] Significantly higher mean serum VEGF level was also found in splenectomized patients when each subgroup of patients compared separately regarding their splenectomy status, this may be due to the fact that patients with splenectomy had higher platelet count which is considered as a source of VEGF, so its rise in splenectomized patients is expected.[36] This also explains the significant positive correlation of serum VEGF with platelet count among the patients, with $P < 0.001$, which was similar to other studies.[30,35] In addition, this significant correlation was also found between serum VEGF and platelet count within each group separately. While no significant correlations were found with other CBC parameters, including Hb level, which may be because those patients were receiving regular blood transfusions.

Although ferritin is a newly identified angiogenic regulator that stimulates the formation of blood vessels by attaching to the antiangiogenic domain of HKa and blocking HKa's actions, no significant correlation was found between VEGF and serum ferritin level, similar to other studies.[30,34,35] This may be due to the effect of iron chelation therapy.

In thalassemic patients, a significant positive correlation was observed between serum VEGF and the age of starting chelation therapy ($r = 0.475$, $P = 0.006$), this agreed with

other studies.[17,30,35] Therefore, early chelation therapy use will aid in reducing angiogenesis. This result differed from that of Farokhi *et al*.[34] They observed no association between serum VEGF and chelation starting age; this may be because the mean age at which iron chelation is started varies, (4.4 ± 1.7) years in our study and (5.21 ± 4.12) years in the other study. Regarding patients with S/β-thal, serum VEGF was negatively correlated with the age of starting chelation therapy, however, this correlation was insignificant. This was not comparable to other studies, possibly due to a very small sample size, since only 8 patients were on chelation therapy in this group. No significant correlation of serum VEGF with the age of starting blood transfusion was found in either patient's subgroups, most studies compared serum VEGF with the frequency of blood transfusion rather than the starting age of transfusion. However, all the thalassemic patients in our study received blood transfusions on a regular basis. There was no significant difference in serum VEGF when was compared in the SCD group according to the frequency of blood transfusion. This was in contrast to Fahmey *et al*.[30] in which, led to the conclusion that routine blood transfusions aid to reduce angiogenesis because there was a strong negative association between VEGF and blood transfusion frequency. The small sample size in our study may be to blame for this discrepancy.

No significant correlation between serum VEGF and the type of chelating drugs used was noticed. These data go in concordance with previous studies.[17,30,34]

Conclusions

β-thal major and S/β-thal patients had significantly higher serum VEGF levels than healthy controls, and splenectomized patients have higher serum VEGF levels than non-splenectomized patients. Furthermore, a significant correlation was seen between serum VEGF level and platelet count in patients with β-thal major and S/β-thal. A significant positive correlation exists between serum VEGF level and the age of starting iron chelation therapy. The levels of serum Ferritin and VEGF did not significantly correlate in either group.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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