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Evaluation of serum vascular cell adhesion marker-1 in β- thalassemia major patients

Nuha Abd Ali Al-Sarai, Subh Salim Al-Mudallal[1], Rana Rai'd Najm[2]

Abstract:

BACKGROUND: Defects in the production of one or more hemoglobin chains result in a set of hereditary hematologic illnesses known as thalassemias. Alpha and β-thalassemia are the two primary thalassemia classifications. The pathophysiology of β-thalassemia is significantly influenced by endothelial activation markers, such as vascular cell adhesion molecule 1 (VCAM-1), which have been found to be higher in both transfusion-dependent and nontransfusion-dependent thalassemia patients. These markers have been suggested for the follow-up of vascular disease in this patient group.

OBJECTIVES: The aim of this study was to evaluate the level of soluble VCAM-1 in β-thalassemia major patients and to correlate its level with the clinical parameters.

PATIENTS, MATERIALS AND METHODS: This case–control study was enrolled on 40 patients with β-thalassemia major. They were collected from Thalassemia Center of Babylon Maternity and Pediatrics Teaching Hospital, from December 28, 2022, to March 30, 2023. Twenty of them were splenectomized, others were not. Another 40 apparently healthy individuals with no family history of thalassemia or other hemoglobinopathy were enrolled in this study as controls. The samples were collected to assay the human VCAM-1 using enzyme-linked immunosorbent assay based on the biotin double antibody sandwich technology.

RESULTS: Among 80 subjects, age and body mass index were similar across groups. There are differences in complete blood count (CBC) parameters between thalassemic and control groups. Thalassemic patients displayed notably higher levels of VCAM-1 and ferritin compared to controls and in splenectomized patients than nonsplenectomized patients. There is a high significant correlation exists between VCAM-1 and ferritin, VCAM-1 and various CBC parameters.

CONCLUSIONS: There are higher levels of VCAM-1 in β-thalassemia major patients compared to the control and in splenectomized compared to non-splenectomized patients which usually associated with disease complications, furthermore it reflect endothelial activation and dysfunction.

Keywords:

Splenectomized, vascular cell adhesion molecule 1, β-thalassemia major

BMT Center, Baghdad Medical City, [1]Department of Pathology, Arab Board for Medical Specialization, Baghdad, [2]Babil Health Directorate, Maternity and Pediatrics Hospital, Hillah, Iraq

Address for correspondence:
Dr. Nuha Abd Ali Al-Sarai, BMT Centre, Baghdad Medical City, Baghda, Iraq.
E-mail: nuha.abdali@gmail.com

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Introduction

Thalassemias are a group of inherited hematologic disorders caused by defects in the synthesis of one or more of the hemoglobin (Hb) chains. The two main categories of thalassemia are alpha and β-thalassemia that are then divided into further subcategories.[1,2] Alpha thalassemia is caused by reduced or absent synthesis of alpha globin chains, disease severity ranges from mild to severe depending on the number of deleted alleles[3] and β-thalassemia is caused by reduced or absent synthesis of β-globin chains therefore imbalances of globin chains occur, ineffective erythropoiesis, hemolysis, and a variable degree of anemia.[4] The overproduction of α-globin tetramers and their interaction with the red cell membrane cause hyperplasia of erythroid precursors and hemolytic

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anemia.[5] In addition to this process, other mechanisms have been shown to cause red cell lysis and exacerbate hemolysis in individuals with β-thalassemia patients.[6]

The clinical manifestations of β-thalassemia are variable, ranging from the transfusion-dependence to silent status of thalassemia trait.[7]

Thalassemia is a hypermetabolic state and produces a chronic effect of unbuffered oxidative stress on the vascular system. Thalassemia patients have impaired endothelial relaxation, intimal thickening, abnormal vascular stiffening, and degeneration of elastic arteries. Premature vascular aging is not solely iron mediated, the cytokines released as a result of inflammatory and infectious processes or directly released by migratory leukocytes, leading to the activation of the endothelium, this results in adherence of erythrocytes to endothelial cells via multiple mechanisms, soluble adhesion molecules (e.g., vascular cell adhesion molecule 1 [VCAM-1]) act as a link to leukocytes and erythrocytes that adhere to the endothelium results in narrowing of the blood vessels.[8]

VCAM-1 (CD106) is a transmembrane glycoprotein expressed only on cytokine-activated endothelium; several studies have shown that VCAM-1 is involved in the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium. It also functions in leukocyte-endothelial cell signal transduction, it plays a role in the development of atherosclerosis, and have been implicated as a mediator of angiogenesis.[9] For the above reasons, this study was design to measure the level of VCAM-1 in Beta thalassemia patients.

Patients and Methods

A case–control study was enrolled 40 patients with transfusion dependent β-thalassemia, 20 males and 20 females with ages ranged between 15 and 30 years.

Diagnosis of thalassemia was done previously and based on the results of Hb electrophoresis, the clinical course of disease and family history of the patients. Patients were subdivided into two subgroups, 20 splenectomized thalassemia major patients, and 20 nonsplenectomized thalassemia major patients.

Patients with any medical history of allergies, positive hepatitis viral screening, acute infection or other hemoglobinopathies were excluded from the study.

A total of 40 apparently healthy individuals, 18 males and 22 females, their age ranged between 15 and 30 years with no family history of thalassemia or other hemoglobinopathy were included as control subjects.

Clinical data of each patient including age, type of thalassemia, blood transfusion frequency, family history, medical history, and drug history were reported from patients by questionnaire. Investigation results of previous complete blood count (CBC), blood film, virology screen, renal function test (RFT), liver function test (LFT), and serum ferritin and reports of abdominal ultrasound and echocardiogram were collected from patients' records.

From each patient, 2 mL of venous blood were collected in K2 ethylenediaminetetraacetic acid anti-coagulant tube for peripheral blood smears preparation using Leishman stain. The total corrected white blood cell (WBC) count acquired using the formula: Total corrected WBC count = total WBC count – (total WBC count × percentage of nucleated red blood cells on differential count/100).[10]

Pretransfusion basic hematological parameters and indices Hb, packed cell volume and platelet count and ferritin levels were taken from the patient's medical file.

Another 1 mL of peripheral venous blood were collected in gel tubes, and allowed to clot for 1 h at room temperature followed by centrifugation for 15 min at 1000 × g. Serum was collected in Eppendorf tubes, then stored at −80°C for 3 months' period, and then used for measuring serum VCAM-1 levels by enzyme-linked immunosorbent assay (ELISA).

Samples from blood donors for control were collected at Babylon central blood bank, 2 mL of venous blood for ferritin and VCAM-1 analysis and their complete blood picture parameters were assessed by automated hematology analyzer.

The method of measurement uses ELISA based on the biotin double antibody sandwich technology to assay VCAM-1. VCAM-1 antigen was added to the wells, which are precoated with anti VCAM-1 monoclonal antibody (the primary antibody), then anti-VCAM-1 antibodies labeled with biotin were added to each well together with streptavidin-HRP enzyme, they were incubated for 1 h at 37°C.

After incubation, the unbound enzymes were removed by washing then substrate A and B were added to each well and incubated again for 10 min. At last, the solution turned blue after incubation, and finally changed into yellow when the stop solution was added (with the effect of acid present in the stop solution). The color shades of the solutions in each well and the concentration of human VCAM-1 are positively correlated. Detection range of VCAM-1 is (0.2 ng/mL → 60 ng/mL).[11]

The standard curve was drawn using computer software capable of generating four-parameter logistic curve.

The curve was plotted on a graph paper, with standard concentration on the X-axis and OD values on the Y-axis as shown in Figure 1. The duplicate readings for each standard and samples were averaged.

Ethical approval

The current study was approved from the scientific committee of Arab council for health specialization as well as approval from Thalassemia Center of Babylon Maternity and Pediatrics Teaching Hospital.

A consent was obtained from each participant before to the collection of the sample.

Statistical analysis

Statistical Package for the Social Science version 26 (IBM Corp., Armonk, NY) program was used to detect the effect of difference factors in study parameters using independent t-test, Pearson correlation, Chi-square, and receiver operating characteristic (ROC).

Results

Statistical characteristics of the study population were analyzed; the normality test of data was performed and revealed normal distributions of different variables in all main and subgroups. There were nonsignificant differences between all groups regarding age and body mass index. There was nonsignificant correlation between sex and subjects' distribution as shown in Table 1.

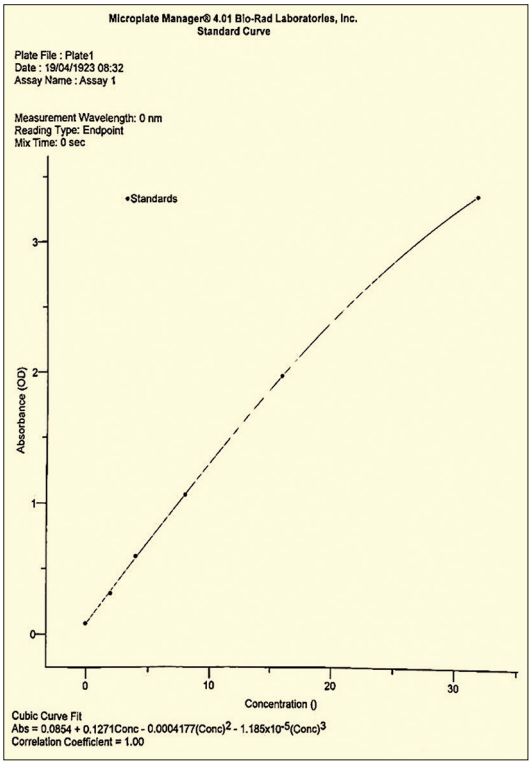

Figure 1: Standards curve of vascular cell adhesion molecule 1 antigen

Thalassemic patients underwent splenectomy had lower frequency of blood transfusion per month. On the other hand, the nonsplenectomized thalassemic patients had high frequency of blood transfusion per month. There is significant correlation between frequency of blood transfusion and subjects' distribution in thalassemic groups was found with $P = 0.011$ [Table 2].

Statistical characteristics of subject's CBC test were studied and comparisons in means of CBC parameters were estimated between study groups [Table 3].

Significant differences in the mean of CBC parameters were found between the thalassemic the control groups, with a $P < 0.01$, except for platelet count, which had a $P = 0.001$ which is highly significant. Furthermore, significant differences in the mean of CBC parameters were found between the splenectomized and the nonsplenectomized thalassemic groups, with $P < 0.01$, except for WBC with a $P = 0.007$, red blood cell count with a $P = 0.005$, and mean corpuscular volume (MCV) ($P = 0.012$).

The study also compared the serum levels of VCAM-1 and ferritin between the studied groups [Table 4]. The concentrations of VCAM-1 and ferritin in the sera of all patients in the thalassemic group and the control group were estimated.

There was a significant increase in the mean serum level of VCAM-1 and ferritin in thalassemic patients compared to the control group using *t*-test [Figures 2 and 3].

Concentration of VCAM-1 and ferritin were estimated in splenectomized group and nonsplenectomized group, and the comparison of VCAM-1 and ferritin concentrations between two subgroups was determined [Table 5].

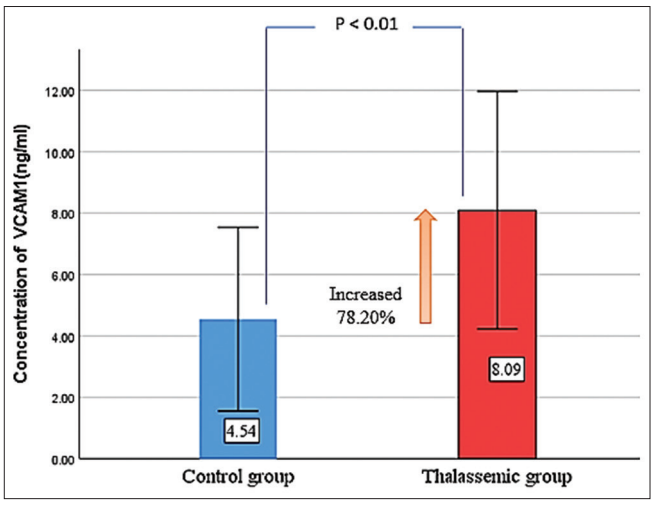

Figure 2: Comparison in serum levels of vascular cell adhesion molecule 1 between study groups. VCAM1 = Vascular cell adhesion molecule 1

Table 1: Statistical characteristics of the study population

Variables	Thalassemic group (*n*=40)	Thalassemic nonsplenectomized (*n*=20)	Thalassemic splenectomized (*n*=20)	*P*	Control group (*n*=40)	*P*
Age (years), mean±SD	18.35±4.94	16.85±4.23	19.85±5.24	0.054 NS	24.10±3.51	0.054 NS
BMI (kg/m^2), mean±SD	20.0±3.82	19.67±4.30	20.34±3.36	0.59 NS	26.20±4.65	0.59 NS
Sex, *n* (%)						
Male	20 (50)	9 (45.0)	11 (55.0)	0.52 NS	18 (45)	0.65 NS
Female	20 (50)	11 (55.0)	9 (45.0)		22 (55)	

BMI=Body mass index, NS=Not significant, SD=Standard deviation

Table 2: Comparison in frequency of blood transfusions between thalassemic splenectomized and thalassemic nonsplenectomized subgroups

Variables	Frequency	Thalassemic nonsplenectomized (*n*=20), *n* (%)	Thalassemic splenectomized (*n*=20), *n* (%)	*P*
Frequency of blood transfusion/month	1	3 (15.0)	10 (50.0)	0.011*
	2	6 (30.0)	8 (40.0)	
	3	6 (30.0)	2 (10.0)	
	4	5 (25.0)	0	

*Significant difference

Table 3: Statistical characteristics of subject's complete blood count test

Variables	Thalassemic group (*n*=40)	Thalassemic nonsplenectomized (*n*=20)	Thalassemic splenectomized (*n*=20)	*P*	Control group (*n*=40)	*P*
WBC 10^3/µL	14.13±4.51	12.25±3.27	16.02±4.86	0.007*	7.25±2.70	<0.01*
RBC 10^6/µL	2.95±0.59	2.70±0.49	3.21±0.58	0.005*	4.45±0.47	<0.01*
Hb (g/dL)	7.49±1.48	6.33±0.98	8.65±0.84	<0.01*	13.52±1.14	<0.01*
PCV (%)	22.74±3.80	20.03±2.54	25.46±2.77	<0.01*	41.23±3.07	<0.01*
MCV (fL)	75.08±6.93	72.40±7.02	77.77±5.85	0.012*	89.95±4.66	<0.01*
MCHC (g/dL)	306.88±18.91	296.47±16.96	317.30±14.77	<0.01*	332.82±30.62	<0.01*
MCH (pg)	23.22±3.27	21.46±2.85	24.99±2.69	<0.01*	30.72±2.38	<0.01*
Platelet 10^6/µL	335.25±59.61	303.40±45.48	367.10±55.59	<0.01*	294.45±46.09	0.001*
RDW (%)	16.79±4.32	14.18±1.80	19.40±4.55	<0.01*	12.83±1.60	<0.01*

*$P < 0.01$ consider significant. WBC=White blood cell, RDW=Red cell distribution width, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCHC=Mean corpuscular hemoglobin concentration, MCH=Mean corpuscular hemoglobin, RBC=Red blood cell, Hb=Hemoglobin

Table 4: Comparison in serum levels of vascular cell adhesion molecule 1 and Ferritin between study groups

	Control group (*n*=40)	Thalassemic group (*n*=40)	*P*
VCAM - 1 (ng/mL)			
Mean±SD	4.54±2.98	8.09±3.86	<0.01*
Minimum–maximum	0.13–10.26	1.90–17.00	
SE	0.47	0.61	
Ferritin (ng/mL)			
Mean±SD	40.05±9.17	3744.80±1727.43	<0.01*
Minimum–maximum	20.0–60.0	552.0–6460.0	
SE	1.45	273.13	

*Significant difference. VCAM - 1: Vascular cell adhesion molecule 1, SE: Standard error, SD: Standard deviation

There was a significant increase in the mean serum level of VCAM-1 in the splenectomized group compared to the nonsplenectomized group, with an approximately 39.90% increase in VCAM-1 level from the mean of the nonsplenectomized group. Similarly, there was an increase of approximately 93.47% in the serum level of ferritin in the splenectomized group compared to the nonsplenectomized group [Figure 4].

As the VCAM-1 level was significantly higher in patients with thalassemia, the discriminatory ability of VCAM-1 between the study groups was estimated using the receiver operating characteristic curve (ROC curve). Figure 5 and Table 6 show the area under curve (AUC) and receiver operator curve of the VCAM-1 in differentiation between thalassemic and control groups.

The cutoff value of 5.4 (ng/mL) represents the optimal point value for discrimination between thalassemic patients and the control group, with a sensitivity of 72.5 and specificity of 65.0. On the other hand, the discriminatory ability of VCAM-1 between the splenectomized and nonsplenectomized groups was estimated using the ROC curve. Figure 6 and Table 7 show the AUC and receiver operator curve of the VCAM-1 in differentiating between splenectomized and nonsplenectomized.

With a sensitivity of 60.0 and a specificity of 55.0, the cutoff value was 7.37 (ng/mL). When distinguishing between thalassemic patients and the control group, the VCAM-1

demonstrated greater sensitivity, specificity, and area under the curve than when differentiating between splenectomized and nonsplenectomized thalassemic patients. However, the cutoff value of VCAM-1 is lower when discriminating between thalassemic patients and control group than when differentiating between splenectomized and nonsplenectomized thalassemic patients.

In the present study, the correlation between different continuous variables in patients with thalassemia was studied [Table 8]. The results showed a strong positive correlation between VCAM-1 and ferritin with a Pearson coefficient of 0.68, and a positive correlation between VCAM-1 and WBC, platelet count, and red cell distribution width, with Pearson coefficients of 0.52, 0.403, and 0.483, respectively. In contrast, there was a weak negative correlation between VCAM-1 and Hb.

Table 9 shows that there is a significant correlation between VCAM-1 and the frequency of transfusions between splenectomized patients with a (P = 0.46), and nonsplenectomized patients with a (P = 0.33).

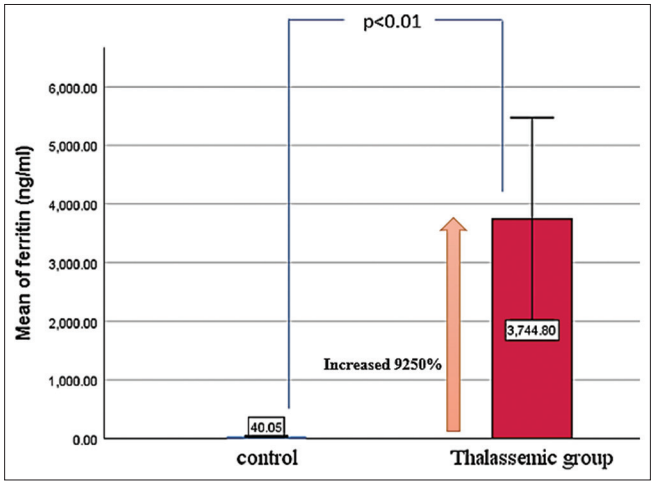

Figure 3: Comparison in serum levels of ferritin between study groups

Discussion

Endothelial injury and inflammation have a profound impact on the pathophysiology of β-thalassemia. The production of adhesion molecules such as VCAM-1 is induced by TNF and other pro-inflammatory cytokines, reactive oxygen species, oxidized low-density lipoprotein, high glucose concentrations, and shear stress, as the endothelium develops to a dysfunctional condition. It has been found that endothelial activation markers are higher in both transfusion-dependent and nondependent subjects,[12] according to researches, VCAM-1 adhesive molecules may serve as an indicator of endothelium alterations.

In comparison to control, thalassemia patients had considerably reduced mean Hb concentrations, MCVs, and mean corpuscular hemoglobin s. This outcome was anticipated because it is an essential part of the disease's pathophysiology. These findings aligned with the research conducted by Abdulsattar *et al*.[1]

In this study, individuals with thalassemia major and control group showed no noticeable variation regarding gender with a P = 0.52 and 0.65, respectively. In addition, considering age the frequency distribution showed no significant variation of age with a P = 0. 54 for patients and control participants.

Forty β-thalassemic patients were evaluated to see whether they had activation of peripheral blood cells and endothelial cells. The patient groups had considerably greater blood ferritin levels, compared to control with a $P < 0.01$, particularly the splenectomized patients $P < 0.01$, due to recurrent infections, inadequate erythropoiesis, and repeated transfusions, an iron excess came to be recognized this is in agreement with other studies done on β-thalassemia major, Mahmoud *et al*.[13] and Alathari *et al*.[3] These results can be explained by the fact that the spleen is a reservoir for iron and that iron from the spleen appears to be eliminated more quickly

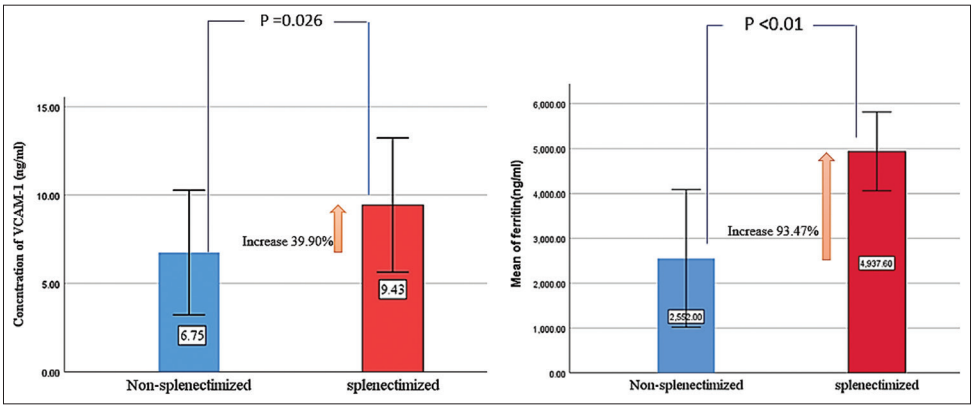

Figure 4: Comparison in serum levels of vascular cell adhesion molecule 1 and ferritin between subgroups. VCAM1 = Vascular cell adhesion molecule 1

Table 5: Comparison in serum levels of vascular cell adhesion molecule - 1 and ferritin between subgroups

	Thalassemic nonsplenectomized (*n*=20)	Thalassemic splenectomized (*n*=20)	*P*
VCAM -1 (ng/mL)			
Mean±SD	6.74±3.52	9.43±3.79	0.026*
Minimum–maximum	1.90–14.37	4.56–17.00	
SE	0.78	0.84	
Ferritin (ng/mL)			
Mean±SD	2552.00±1535.71	4937.60±878.27	<0.01*
Minimum–maximum	552.0–5800.0	3549.0–6460.0	
SE	343.39	196.38	

*Significant difference. VCAM - 1=Vascular cell adhesion molecule 1, SE=Standard error, SD=Standard deviation

Table 6: Discriminatory ability of vascular cell adhesion molecule - 1 between patient group and control group

	Sensitivity	Specificity	AUC (%)	Cutoff (ng/mL)
VCAM-1	72.5	65.0	76.0	5.40

VCAM - 1=Vascular cell adhesion molecule 1, AUC=Area under curve

Table 7: Discriminatory ability of vascular cell adhesion molecule - 1 between thalassemic splenectomized and thalassemic nonsplenectomized

	Sensitivity	Specificity	AUC (%)	Cutoff (ng/mL)
VCAM-1	60.0	55.0	70.0	7.37

VCAM - 1=Vascular cell adhesion molecule 1, AUC=Area under curve

Table 8: Correlation between complete blood count parameters and vascular cell adhesion molecule - 1 in patient's group

Correlation test	VCAM-1		
	Pearson correlation	*P*	R^2
Ferritin	0.68	<0.01*	0.468
Hb	–0.37	0.021*	0.142
WBC	0.52	0.001*	0.274
Platelets	0.403	0.01*	0.162
RDW	0.483	0.002*	0.233

*Significant correlation. VCAM - 1=Vascular cell adhesion molecule 1, WBC=White blood cell, RDW=Red cell distribution width, Hb=Hemoglobin

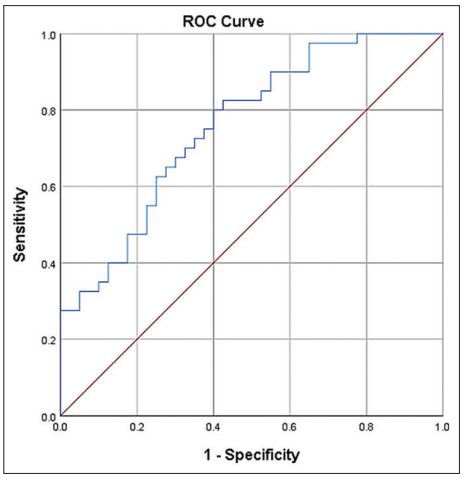

Figure 5: The discriminatory ability of vascular cell adhesion molecule 1 between patient group and control group. ROC = Receiver operating characteristic

than iron from the liver when utilizing an efficient chelation treatment.[14]

In comparison to the controls, the study found that all *β*-thalassemics had substantially higher blood levels of VCAM-1, confirming their overexpression, and suggesting activated endothelial cells. These findings are in line with an earlier research[15] which suggested that iron excess, frequent blood transfusions, and pathogen exposure are all associated with the cellular activation seen in β-thalassemia patients.

Serum VCAM-1 levels were found to be higher in splenectomized β-thalassemics than in nonsplenectomized b-thalassemics with a $P = 0.026$, indicating a higher endothelial activation this finding implies that splenectomized patients may have a higher risk for experiencing thromboembolic manifestations as a long-term complication, as reported by other studies.[16]

Once more, ferritin levels were observed to positively correlate with serum VCAM-1 with a $P < 0.01$. The relationship between ferritin and activation molecules in β-thalassemics suggests that an excess of iron can be responsible for endothelial damage, atherogenesis, and a higher risk of thrombosis.[17]

Moreover, blood transfusion frequency in the thalassemic subgroups was shown to be significantly correlated. P <0.01 as serum s. VCAM-1 levels were significantly lower in nonsplenectomized thalassemics. The considerable difference in serum sVCAM-1 levels between splenectomized and nonsplenectomized thalassemics can be attributed to the frequent blood transfusions in nonsplectomized patients.[18]

Chronic transfusion treatment has a well-known effect on VCAM-1 expression as documented by a number of studies, it was suggested that blood transfusions might reduce pro-coagulant activity, aiding in the prevention of vascular complications also, VCAM-1 antigen showed increased sensitivity, and specificity when discriminating thalassemic patients from the control group, in agreement with another research.[17] Therefore, the level of VCAM-1 molecule can be an essential marker and a useful test to predict and assist in the diagnosis of

Table 9: Correlation between vascular cell adhesion molecule - 1 and frequency of blood transfusion between thalassemic splenectomized and thalassemic nonsplenectomized patients

Variables	Subgroup	Frequency of blood transfusion/month				*P*
		Frequency (1)	Frequency (2)	Frequency (3)	Frequency (4)	
VCAM (ng/mL), mean±SD	Thalassemic nonsplenectomized	7.06±2.96	5.85±2.52	7.20±4.43	7.07±4.50	0.46 S
	Thalassemic splenectomized	9.39±4.23	9.51±3.68	9.27±4.18	9.43±3.79	0.33 S

S: Significant correlation, VCAM - 1=Vascular cell adhesion molecule 1, SD=Standard deviation

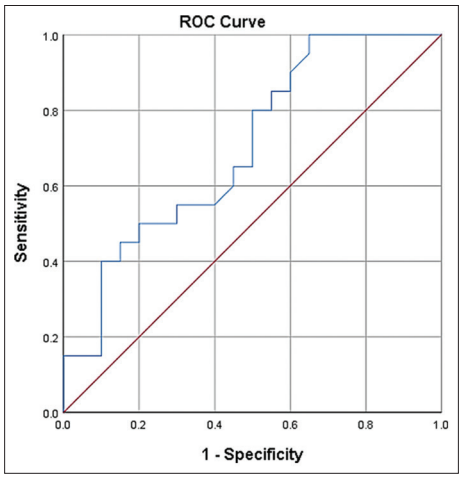

Figure 6: The discriminatory ability of vascular cell adhesion molecule 1 between thalassemic splenectomized and thalassemic nonsplenectomized

complications and hemostatic changes. Its level can be thought of as a useful signal for thalassemic patients, to monitor and follow-up for early and appropriate action to reduce complications.[17]

Conclusions

There are higher levels of VCAM-1 in β-thalassemia major patients compared to the control and in splenectomized compared to non-splenectomized patients which usually associated with disease complications, furthermore it reflect endothelial activation and dysfunction that occur in thalassemic patients.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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