Evaluation the Level of VCAM-1 in Patients with Sickle Cell Disease as Marker for Endothelial Dysfunction and Associated with Coagulation Factors and Some Blood Parameters in both Adults and Childs,'

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ABSTRACT

Sickle Cell Anemia is a genetic disease resulting from the exchange of amino acids in the hemoglobin protein, which carries oxygen in the blood, transforming the natural disk shape of red blood cells into the sickle-like shape.

Objective we aimed to study the role of VCAM-1 as a marker of endothelial dysfunction in SCA patients. Evaluate the association between serum sVCAM-1 level in SCA patients, and the frequency of vaso-occlusive crises. Possible usage of these markers as a predictive index for VOC occurrence. Study correlations between sVCAM-1 with different hemolysis and inflammation markers.

Patients and methods The total number of participants are (100) person including (35) patients with painful crisis, (35) patients with steady state, and (30) healthy control group, all of the patients and healthy control group were male, in addition, Their ages ranged from (3-55).were evaluated with respect to serum vcam-1 level by means of enzyme-linked immune sorbent assay.

Results The results showed there have been significant differences in concentrations of Vcam-1, D dimer, and CRP in SCA patient (painful crisis, steady state) in comparison with the control group for both adults and children.
The results also showed that there were significant differences in platelet, and PCT levels between patients with sickle cell anemia and healthy subjects, in adults only, but in children, there were no significant differences between patients with sickle cell anemia and healthy group.
In this study, SCA patients with the painful crisis and steady state have significant correlations between (vcam-1, D-dimer),

Keywords: sickle cell disease, vaso-occlusive crises, Vcam-1

الملخص فقر الدم المنجلي هو مرض وراثي ناتج عن تبادل الأحماض الأمينية في بروتين الهيموجلوبين الذي يحمل الأكسجين في الدم، فيتحول شكل القرص الطبيعي لخلايا الدم الحمراء إلى الشكل المنجلي.

الهدف نهدف إلى دراسة دور VCAM-1 كعلامة على خلل بطانة الأوعية الدموية لدى مرضى SCA. تقييم الارتباط بين مستوى sVCAM-1 في الدم لدى مرضى SCA، وتواتر أزمات انسداد الأوعية الدموية. إمكانية استخدام هذه العلامات كمؤشر تنبؤي لحدوث ازمات انسداد الاوعية الدموية. دراسة الارتباطات بين VCAM-1 مع انحلال الدم وعلامات الالتهاب المختلفة.

المرضى وطرق العمل بلغ عدد المشتركين (100) شخص منهم (35) مريضا يعانون من أزمة مؤلمة، (35) مريضا يعانون من حالة مستقرة، و (30) مجموعة الاصحاء، جميع المرضى ومجموعة الاصحاء كانوا من الذكور، في بالإضافة إلى ذلك، تراوحت أعمارهم بين (3-55). تم تقييم مستوى vcam-1 في مصل الدم عن طريق جهاز الايلايزا.

النتائج أظهرت النتائج وجود اختلافات معنوية في تراكيز VCAM-1 وD dimer وCRP لدى مرضى SCA (حالة أزمة الالم، الحالة مستقرة) مقارنة مع مجموعة الاصحاء لكل من البالغين والأطفال.

كما أظهرت النتائج وجود فروقات معنوية في مستويات الصفائح الدموية ومستوى PCT بين مرضى فقر الدم المنجلي والأصحاء، لدى البالغين فقط، أما عند الأطفال، فلم تكن هناك فروق ذات فروقات معنوية بين مرضى فقر الدم المنجلي ومجموعة الأصحاء.

في هذه الدراسة، كان لدى مرضى SCA الذين يعانون من أزمات الألم والحالة المستقرة ارتباطات كبيرة بين (vcam-1، D-dimer).

INTRODUCTION

Sickle Cell Anemia: is a genetic disease resulting from the exchange of amino acids in the hemoglobin protein, which carries oxygen in the blood, transforming the natural disk shape of red blood cells into the sickle-like shape (1),
Standard RBCs have a spherical form and are compact and resilient, which makes it easy for them to pass through narrow capillaries (2). Low oxygen tension promotes the sickling of red blood cells and repeated episodes of sickling damage the cell membrane and reduce its flexibility, in sickle cell disease. When normal oxygen tension is restored, these cells fail to return to their original form, due to their rigidity, these blood cells cannot deform as they pass through narrow capillaries, which causes vascular blockage and ischemia (3).

SCA was first described by Herrick in 1910 when JAMES B.HERRICK described a case of anemia in a dental student from Grenada that was associated with "peculiar elongated and sickle-shaped red blood corpuscles" on microscopy (4).

The inheritance of sickle cell anemia occurs via an autosomal recessive gene with both parents, in general, asymptomatic individuals who carry a single affected gene (heterozygous), transmitting the defective gene to their child, who therefore is homozygous (Hb SS) (5). The discovery of hemoglobin S (HbS) by Linus Pauling and colleagues in 1949 was the first demonstration that the production of an abnormal protein could be the cause of a genetic disorder (6).

In 1957, Ingram discovered that HbS was caused by a single amino acid substitution (glutamic acid changed to valine) at position 6 of the β-globin chain of hemoglobin, and in 1963, Goldstein showed that this amino acid substitution arose from a single base change (A>T) at codon 6 (rs334). SCA, therefore, has always been at the forefront of molecular medicine and arguably launched the whole field of human molecular genetics (4).

This type of hemoglobin is produce by replacing glutamic acid instead of valine in position six of the β-chain on chromosome 11, which further produces deoxygenation-induced polymerization and finally results in the abnormal crescent shape of red blood cells (RBCs). Also, this sickled hemoglobin makes RBCs vulnerable to broken easily resulting in extravascular and intravascular hemolysis with a result of low hemoglobin levels (7).

The polymerization of deoxygenated hemoglobin S (HbS) together with the formation of irreversibly sickled erythrocytes ending with vaso-occlusion underlies the pathophysiology of sickle cell disease (SCD) and leads to the two hallmarks of the disease: recurrent episodes of acute pain and chronic organ damage (8). Hemoglobin polymerization is connected with different pathophysiological fashion, including; bone crisis,vascular crisis, and aplastic crisis, the hemoglobin polymerization was related to an iron and water content decrease (cell dehydration), and increased erythrocytes density with persistent membrane damage, hemolysis and improved blood flow (9).

SCA is prevalent in areas of Africa, the Mediterranean, the Middle East, India, the peninsula, and Southeast Asia. (10), the United States, and South or Central America (11). The greatest burden of sickle cell anemia (SCA) is in sub-Saharan Africa (SSA), where 75% of the 300,000 global births of affected children live, and estimates suggest that 50–80% of these patients will die before adulthood (12).

In Iraq, Sickle cell disorders, are less uniformly distributed with carrier rates varying from 0 to 16.0%, and they cluster in the extreme north and south of the country (13). the prevalence of sickle cell disease hemoglobin was low 0.56%, which is not representing the true figure of sickle cell carriers in the Karbala governorate because there is no definitive cure for these genetic conditions (10).

Vascular Cell Adhesion Molecule-1 VCAM-1 is a 90-kD a glycoprotein that is inducible and predominantly expressed in endothelial cells. In 1989, VCAM-1 was first recognized as a cell adhesion molecule, that helps regulate inflammation-associated vascular adhesion and the trans endothelial migration of leukocytes, such as macrophages and T cells (14).

Several studies show that levels of soluble VCAM-1 increased in sickle cell anemia patients. VCAM-1 is among the most important inflammatory factors and plays a major role in sickle cell anemia. It is a useful marker for SCA management, as they are endothelial surface activation indicators can be connected with the disease's severity (15).

- **Diagnostic types of sickle cell anemia:** by hemoglobin electrophoresis for both adults and children.
- **Exclusion criteria**

Patients were exclude from the study if they had any of the following:

(1) Acute illness or fever.

(2) Malignancy.

(3) Hepatic or renal dysfunction.

(4) Autoimmune disease or cardiopulmonary disease.

(5) Surgery or trauma within a month before the study.

- **Inclusion Criteria**

1. SCA patients who served as case group.
2. Healthy subject, who served as control group.

- **Body Mass index (BMI)**

Calculated from the following equation
BMI = Weight (kg) / Height (m2)

Statistical analysis:

The version 24 of the computer program SPSS, ANOVA table – one way, R-test and the results have been considered to have statistical significance at ($p \leq 0.05$).

Ethical Management of Studies:

This study was approve by Ethical Committee at College of Applied Medical Science/ University of Karbala. All subjects involved in this work were informed and agreement was obtain verbally from each one before the collection of samples. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 3 (including the number and the date in 1/11/2022) to get this approval.

Patients and methods:

Patients:

The total number of participants are (100) person including 35 patients with painful crisis (adults 19, children 16), 35 patients with steady state (adults 10, children 25), and 30 healthy control group (adults 20, children 10), all of the patients and healthy control group were male, in addition, their ages ranged from (3-55). The patients and healthy subjects were divided into 6 age groups ,less than 10 years, (11-20), (21-30), (31-40), (51-60), and (41-50).

Methods:

soluble adhesion molecule measurements

Soluble adhesion molecules include **soluble vascular cell adhesion molecule-1 (sVCAM-1)** were characterized using ELISA Kits according to the manufacturer's recommendations.

- **Estimation of the level of D-dimer**

D-Dimer concentrations were measure automatically using the VIDAS® D-Dimer Exclusion IITM (DEX2).

- **Estimation of the level of C-Reactive Protein (CRP)**

C - Reactive Protein (CRP) was measure automatically by HIPRO Equipment.

- **Complete Blood Count (CBC)**

CBC was measure automatically by SWELAB Equipment.

RESULTS:

1.1. Soluble Vascular Cell Adhesion Molecule (Vcams-1):

Referring to table (1-1)'s findings, both the adult and child patient groups in comparison to the control showed significant differences (P≤ 0.05). In the same table revealed Vcam-1 level higher in all patient groups (painful crisis and steady state) compared to control.

Table 1-1: Concentration of Vcam-1 in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	1.36 ± 0.36 [ab] (N=26)	2.32 ± 0.19 [a] (N=16)	0.41 ± 0.20[b] (N=10)	0.0000 *	1.458
Adults	1.73 ± 0.31 [b] (N=9)	2.79 ± 0.16 [a] (N=19)	0.95 ± 0.33 [c] (N=20)	0.0000 *	0.458
Total	1.46 ± 0.38	2.57 ± 0.29	0.83 ± 0.38		

* Means significant differences at P ≤ 0.05 NS: no significance

The data in table (1-1) showed that, as compared to the control group, the mean Vcam-1 values of SCA patients significantly increased. Numerous academics have characterized sickle cell disease as a chronic inflammatory condition that is associated with a number of factors, including an increase in the production of reactive oxygen species, hemolysis, endothelium damage, and pro-inflammatory cytokines all these facters lead to released VCAM-1 in high levels in patient with SCA (16). The findings of the current analysis, which demonstrate that Vcam-1 levels were higher in SCA patients (painful crises and steady state) than in controls, are addressed and supported by a number of studies (17).

1.2. C-Reactive Protein (CRP):

Table (1-2) showing that CRP is higher in SCA patient (painful crisis and steady state) compared with control group in both child and adult .There are significant differences between adults, child in both patient groups compared with control (P ≤ 0.05).

Table 1-2: Concentration of CRP in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	1.64 ± 1.30 [b] (N=26)	25.95 ± 42.15 [a] (N=16)	1.05 ±0.47 [b] (N=10)	0.008 *	8.067
Adults	3.01 ± 3.97 [b] (N=9)	43.29 ± 37.78 [a] (N=19)	0.95 ± 0.46 [b] (N=20)	0.0000 *	30.915
Total	2.00 ± 2.30	35.36 ± 40.20	0.98 ± 0.45		

* Means significant differences at P ≤ 0.05 NS: no significance

The data presented in table (1-2) showed that the patient groups' CRP concentrations were significantly higher than those of the control group. Sickle cell diseases has been described by many scholars as a chronic inflammatory disease that is linked to several factors such

as, increased synthesis of reactive oxygen species, hemolysis, endothelial destruction, and increased synthesis of pro-inflammatory cytokines among others. Inflammatory processes play a significant role in the activation of the acute painful vaso-occlusion crisis, which is the primary cause of sickle cell anemia patients' hospitalization (16). Inflammation among sickle cell disease patients is a ceaseless process that occurs even during a steady state (18). These results are in agreement with certain studies that reported an increase in CRP levels. Studies have assessed its value in SCD. high levels of SCA in the steady state have been correlated with increased frequency of acute pain in children with SCD Most studies find that levels increase during acute vaso-occlusion (19).other study showing that C-reactive protein (CRP) levels, was significantly different during VOC compared with the previous steady state values in both adults and child (20).

1.3. D-Dimer:

In table (1-3) Mean concentrations of D-dimer, is higher in the SCA patient (painful crisis and steady state) if compared with control group. There are significant differences between (child and adult) in all patient group compared with control ($P \leq 0.05$).

Table 1- 3: Concentration of D.dimer in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	446.0 ± 109.27 [b] (N=26)	1040.1 ± 145.87[a] (N=16)	73.0 ± 17.77 [c] (N=10)	0.0000 *	4.957
Adults	811.6 ± 150.80 [b] (N=9)	1930.2 ± 549.5 [a] (N=19)	199.9 ± 64.32 [c] (N=20)	0.0000 *	9.687
Total	540.02 ± 201.05	1523.34 ± 609.6	170.61 ± 78.61		

* Means significant differences at $P \leq 0.05$ NS: no significance

In this study's SCA patients, the serum D-dimer level was considerably greater than in healthy controls. Elevated plasma D-dimer levels indicate increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation these increasing are features of steady-state sickle cell disease, and that they further increase during painful crisis. The results of the agreement study (21) are consistent with recent findings that showed greater serum D. dimer levels in both crisis and steady state conditions. The level of D-dimer was significantly higher in of the patients; and significant elevation was observe in patients with VOC when compared with steady state.

1.4. Retic count:

In table (1-4) Mean concentrations of Retic count, is higher in the SCA patient (painful crisis and steady state) if compared with control group. There are significant differences between (children and adults) in all patient groups compared with control ($P \leq 0.05$).

Table 1-4: Concentration of Retic count in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	3.69 ± 4.11 [b] (N=26)	7.39 ± 4.26 [a] (N=16)	2.63 ± 2.41 [c] (N=10)	0.009 *	0.770
Adults	2.99 ± 4.44 [ab] (N=9)	5.48 ± 4.64 [a] (N=19)	1.69 ± 1.01 [c] (N=20)	0.006 *	3.412

Total	3.51 ± 4.14	6.35 ± 4.51	1.91 ± 1.45		

* Means significant differences at P ≤ 0.05 NS: no significance

These findings were consistent with other previousiy published data in which authors reported that Retic count higher in painful crisis, steady state compared with control groups. Due to the fact that adult erythrocytes have a 120-day lifespan, mature erythrocyte Hb cannot sensitively reflect Hb synthesis. While this is happening, reticulocytes are released into the peripheral blood from the bone marrow, and they later develop into mature erythrocytes in the following 1–2 days, this leading to increase in Retic count.The difference in mean of Retic count was statistically significant (p≤ 0.05) in both children and adults (22).

1.5. Platelet:

Table (1–5) displays the findings of the platelet levels in both the patient and control groups. We found significantly higher concentration of platelets in SCA patients (painful crisis and steady state) as compared to the control group (P≤0.05).

Table 1-5: Concentration of Platelet in patients with SCA and compare with control group

Categories	**Mean ± SD**			***P value***	**LSD**
	Patients (70)		**Healthy (30)**		
	Steady (35)	**Painful (35)**			
Children	329.8 ± 237.6 (N=26)	315.5 ± 200.8 (N=16)	289.5 ± 161.4 (N=10)	0.916	NS
Adults	390.0±155.8 [a] (N=9)	260.36±150.4 [b] (N=19)	230.25±48.5 [b] (N=20)	0.006 *	119.68
Total	345.28±219.01	285.6 ± 174.82	243.92 ± 87.5		

* Means significant differences at P ≤ 0.05 NS: no significance

Only in adults did the results of table (1-5) show statistically significant variations in platelet concentrations between SCA patients and controls, A higher mean platelet concentration signifies a tendency to hypercoagulability and thromboembolic complications. Autosplenectomy associated with Sickle Cell Diseases which causes reduced or absent splenic sequestration could account for thrombocytosis seen in the disease. This result agreement with other study that found the mean of platelet values both in VOC and steady state were significantly lower than the controls values in adults and children. The sickle cell anemia patients are continually hemolysing their red cells with a short survival rate of the erythrocytes between 12-14 days and as a result, the hemoglobin, hematocrit, platelet and red blood cells are usually lower than normal healthy individuals (23). Our study disagreement with this study in children.

1.6. Hemoglobin (HB):

When compared to the control group, the results of HB in table (1–6) showed a significant rise (P 0.05) in Hb concentration in SCA patients. The level of hemoglobin in SCA patients (painful crisis, steady state) is higher if compared with and the control group.

Table 1-6: Concentration of HB in patients with SCA and compare with control group

Categories	**Mean ± SD**			***P value***	**LSD**
	Patients (70)		**Healthy (30)**		
	Steady (35)	**Painful (35)**			
Children	9.59 ± 1.50 [b] (N=26)	8.65 ± 1.69 [c] (N=16)	12.11 ± 2.18 [a] (N=10)	0.0000 *	0.459
Adults	8.53 ± 2.11 [c] (N=9)	10.20 ± 1.77 [b] (N=19)	14.68 ± 1.88 [a] (N=20)	0.0000 *	0.556
Total	9.32 ± 1.71	9.49 ± 1.88	14.09 ± 2.20		

* Means significant differences at P ≤ 0.05 NS: no significance

The data in table (1-6) showed a significant decrease in HB concentration in the patient groups in the case when compared with control groups. Sickled hemoglobin makes RBCs vulnerable to broken easily resulting in extravascular and intravascular hemolysis with a result of low hemoglobin levels in patient groups comparing with control group These results are in line with several studies, the hematological indices during crisis and steady state showed that the mean hemoglobin in VOC were significantly lower than the mean steady state and the controls (23).

1.7. Platelatcrit (PCT):

Table (1-7) showing that is PCT higher in SCA patient (painful crisis and steady) compared with control group in both child and adult. There are no significant differences between child in both patient groups and control but there are significant differences between adult in both patient groups and control (P ≤ 0.05).

Table 1-7: Concentration of PCT in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	0.257 ± 0.19 (N=26)	0.208 ± 0.14 (N=16)	0.200 ± 0.12 (N=10)	0.599	NS
Adults	0.322 ± 0.14 [a] (N=9)	0.190 ± 0.11 [b] (N=19)	0.185 ± 0.08 [b] (N=20)	0.008 *	0.048
Total	0.274 ± 0.18	0.198 ± 0.13	0.188 ± 0.09		

* Means significant differences at P ≤ 0.05 NS: no significance

The results of the statistical analysis for PCT, as shown in table (1-7), showed no significant differences in the PCT value between SCA patients and controls among children, the mean of PCT for SCA patients as compared to adult controls showed a significant increase (P ≤ 0.05) in the same table, The higher PCT count seen in patients with SCA could be attributed to a possible splenic sequestration, reduction or absence of spleen resulting from hyposplenism in SCA or autosplenectomy, as well as the underlying chronic inflammation. this results agreement with other studies that found the value of PCT increase in SCA patient (painful crisis, steady state) compared to control group with out any significant differences in children (24). But our results disagreement with these study in adults because we found significant differences between SCA patient and control in adults due to the change in geographical area, environmental factors, sample size, and age differences.

CORRELATION:

Table 1-8: Correlation between markers

Correlation between markers	R2
Correlation between Vcam-1 and D-dimer in steady patients group	0.407

Correlation between Vcam-1 and D-dimer in painful patients group	0.511

According to the results in table (1-8) there were significant correlation between Vcam-1 and D-dimer in SCA steady patients group in both children and adults (R2 = 0.407). This indicates that the rise in D. dimer is associated with the rise in VCAM-1 in steady patients group. This indicates that the rise in D-dimer is associated with the rise in Vcam-1. According to the results of this study (21) D-dimer level Elevated due to the increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation these increasing are features of steady-state sickle cell disease that lead to endothelial dysfunction ending with released VCAM-1 in high level.

While in table (1-8) there were significant correlation between VCAM-1 and D-dimer in SCA painful patients group in both children and adults (R2 = 0.511). When D-dimer levels increase VCAM-1 levels will be increase. Depending on the results of this study (21) D-dimer level Elevated due to the increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation these increasing during painful crisis sickle cell disease that lead to endothelial dysfunction ending with released VCAM-1 in high level. These results are in agreement with several studies' suggestions that there are present significant correlations between soluble vascular cell adhesion molecule-1 and D-dimer in painful patients group (25).

CONCLUSIONS:

The level of Vcam-1 is higher in patients compared to healthy subjects, with a positive relationship between Vcam-1 level and endothelial dysfunction.

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