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Effects of Hydroxyurea on Some Adhesion Molecules in Sickle Cell Anemia Patients

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Abstract

Sickle cell anemia (SCA) is a genetic disorder in the blood that is characterized by progressive organ malfunction, painful vaso-occlusive crises, and severe hemolytic anemia. This case-control aimed to compare study of this study is to determine the expression level of adhesion molecules: Vascular Cellular Adhesion Molecule-1 (VCAM-1), Intracellular Adhesion Molecule-1 (ICAM-1), Platelet selectin (P-selectin), and Endothelium selectin (E-selectin) in plasma samples using ELISA technique. This research included 78 males and females, 22 of whom were control individuals, and the rest were divided into 2 groups: steady-state sickle cell patients (SCA), and patients under hydroxyl-urea treatment (SCAHU). The study results indicated that plasma E- and P-selectin expression levels were significantly higher in the SCA group when compared to control groups, but there were no significant differences between the SCAHU and SCA groups for both E and P selectins. There was an increase in the ICAM-1 and VCAM-1 levels in the patient groups when compared to the control group, but they were not statistically significant. Our findings suggest that high expression of P-selectin, E-selectin, VCAM-1, and ICAM-1 predisposes patients to severe manifestations of SCA.

Disciplinary: Bioscience & Biochemistry, Medicine & Health Science, Biomedical and Biotechnology.

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1 Introduction

Sickle cell anemia (SCA) is a genetic disorder inflammatory disease [1]. It is one of the most common diseases globally. It is estimated that the hemoglobinopathy trait is carried by 5% of the world's population [2]. This disease is caused by a *β-globin* gene mutation where the 17th nucleotide is altered to adenine from thymine meaning in the *β-globin* chain the 6th amino acid changed from glutamic acid to valine and this leads to the production of an altered form of hemoglobin, known as hemoglobin S (HbS) [3].

SCA is characterized by severe progressive organ malfunction, hemolytic anemia, and painful vaso-occlusive crises (VOC). In addition, patients with this disease are exposed to a chronic inflammatory state and reduced quality and length of life [1]. The critical event in the SCA pathophysiology is the polymerization of HbS, which leads to vaso-occlusion and hemolysis due to physicochemical alterations in the erythrocytes [4].

Adhesion molecules are proteins that are expressed on the cell surface, they mediate the reaction between cells and the extracellular matrix or between other cells. They are divided into 4 groups: cadherins, immunoglobulin-like adhesion molecules, selectins, and integrins. Cell adhesion is vital to maintain the structure and normal function of tissues. It has a crucial role in providing an effective immune response against external pathogens [5]. The intercellular adhesion molecule-1 (ICAM1-1) belongs to the immunoglobulin family and can be expressed by many cell types such as endothelial cells and leukocytes, and can be induced by various kinds of cytokines [6]. Vascular cell adhesion molecule-1 (VCAM-1) is expressed on activated endothelial cells and is an immunoglobulin-like adhesion molecule. It binds to integrin α4β1, which is expressed constitutively on eosinophils, monocytes, and lymphocytes [7].

The selectin family consists of 3 members; they were named after the cell type they were identified in first, being L-selectin (lymphocytes), E-selectin (endothelium), and P-selectin (platelets) [8]. Their role in inflammation as leukocyte trafficking, thrombosis, and cancer metastasis has been identified after the successful cloning of selectins [9]. The function of selectin is to mediate the adhesion of cancer and hematopoietic cells to the platelets, endothelial cells, and leukocytes in the blood [10]. It has been shown that selectin-directed therapeutic agents are effective at blocking many of the pathological effects that result from the entry of leukocytes into inflammation sites [11].

During endothelial disorders, cytokine and chemokine production is elevated in addition to several other complications, including an increase in reactive oxygen production, platelet aggregation, and adhesion molecule expression, including VCAM-1, ICAM-1, and E-selectin [12].

Hydroxyurea (HU) is a drug that is taken orally. Biologically, HU therapy increases fetal hemoglobin HbF levels and decreases the mean corpuscular hemoglobin concentration, the number of irreversibly sickled red cells, hemolysis, leukocyte adhesion, and the red cell adhesion molecules expression [13].

This study determines the level of adhesion molecule expression (VCAM-1, ICAM-1, P-selectin, and E-selectin) in SCA patients with and without HU treatment and compared to healthy control patients using Saudi SCA patients to understand the potential role of these molecules in disease progression and the effects of HU.

2 Materials and Methods

2.1 Human Subjects

After obtaining ethical approval (Reference No 243-19) from King Abdulaziz University Hospital, Jeddah, Saudi Arabia, the study procedure was approved by the Unit of Biomedical Ethics committee from King Abdulaziz University.

This is a case-control study including 78 males and females. Samples were collected from patients: 56 with sickle cell anemia, 28 in steady-state (SCA), 28 taking HU (SCAHU), and 22 of the general (healthy) population included as a control group. Written informed consent and completed clinical questionnaires were obtained from all patients and controls. In addition, clinical data were collected from patient questionnaires and a review of their medical files.

Blood samples were collected from the patients (5 mL of venous blood) in EDTA tubes, and plasma samples were then separated. The following parameters were measured: ICAM-1, VCAM-1, E-selectin, and P-selectin using ELISA kits (sandwich-ELISA).

2.2 Detection of Adhesion Molecules

The following ELISA kits were used in the study: human ICAM-1 (intercellular adhesion molecule 1) ELISA kit catalog no: E-EL-H2585 96T, human VCAM 1/CD106 (Vascular Cell Adhesion Molecule 1) ELISA kit catalog no: E-EL-H5587 96T, human SELP (P-selectin) ELISA kit catalog no: E-EL-H0917 96T, and human SELE (E-selectin) ELISA kit catalog no: E-EL-H0876 96T.

3 Data analysis

For statistical analyses, the statistical package for social science (IBM SPSS Version 22) was used. Data were expressed as mean ± SEM. To compare the data, the Kruskal-Wallis test was used to compare more than 2 groups together, whereas the Mann-Whitney U test was used when comparing 2 groups only. P-values less than 0.05 were considered to be statistically significant.

4 Results

Table 1 shows the patients' demographic characteristics. The statistical analysis data showed that there were no significant differences in the levels of plasma ICAM-1 (Figure 1) and VCAM-1 (Figure 2) between the control, SCA, and SCAHU groups. However, although no significant difference was detected, both the SCA and SCAHU groups showed a higher expression than the control group, which was more evident in the SCA patients.

The data also showed that plasma E-selectin (Figure 3) and P-selectin (Figure 4) levels were significantly higher in the SCA group compared to control groups (P-value = 0.0005 and P-value = 0.0001, respectively). In addition, a significant difference was reported between the SCAHU and the control groups for E-selectin (P-value < 0.0002) and P-selectin (P-value = 0.0002), while no significant differences were found between the SCA and SCAHU groups.

In Figure 1, the data show that there are no significant differences in the level of the plasma ICAM-1 between the three groups as the P-value=0.279. The highest level of ICAM-1 was recorded in SCA patients. Data were expressed as mean ± SEM. Significance between groups was made using a nonparametric test (Kruskal-Wallis test).

Table 1: Characteristics and hematological parameters for the participants.
WBC = white blood cells, RBC = red blood cells.

Parameters	Control (n=22)	SCA (n=26)	SCAHU (n=30)
	A	B	C
Age (years)	28.3 ± 5.2	29 ± 9.2	26 ± 7.9
Male/female	7/15	16/10	12/18
WBC (10^3 /µL)	5.4 ± 1.2	11.4 ± 4.7	11.8 ± 3.4
RBC (10^6 /µL)	4.5 ± 0.6	3.2 ± 0.9	2.4 ± 0.2
Hemoglobin (Hb) [g/dl]	12.8 ± 1.2	8.8 ± 1.2	7.5 ± 0.7
Hematocrit (HCT) [%]	38.2 ± 3.2	26.7 ± 4.6	21.9 ± 1.8
Mean corpuscular hemoglobin (MCH) [pg]	27.9 ± 2.8	27.9 ± 4.5	30.5 ± 2.1
Mean corpuscular volume (MCV) [fl]	83.2 ± 6.8	93.3 ± 9.7	89.3 ± 6.4
Platelets (10^3/µL)	287.7 ± 45.6	515.8 ± 142.5	342.6 ± 144.6
Neutrophils (10^3/µL)	2.5 ± 0.7	5.53 ± 2.5	5.9 ± 2.1
Lymphocytes (10^3/µL)	2.2 ± 0.5	4.66 ± 2.6	4.01 ± 0.9
Monocytes (10^3/µL)	0.5 ± 0.1	0.99 ± 0.46	1.1 ± 0.4
Eosinophil (10^3/µL)	0.17 ± 0.12	0.38 ± 0.27	0.4 ± 0.4
Basophil (10^3/µL)	0.04 ± 0.02	0.085 ± 0.04	0.1 ± 0.08

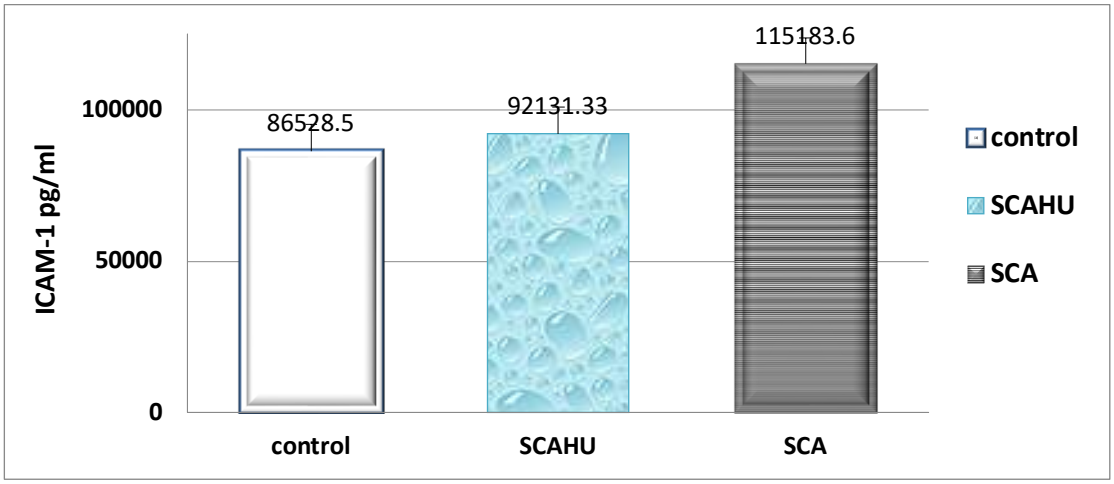

Figure 1: Comparison of plasma levels of ICAM-1 in control, SCA, and SCAHU groups.

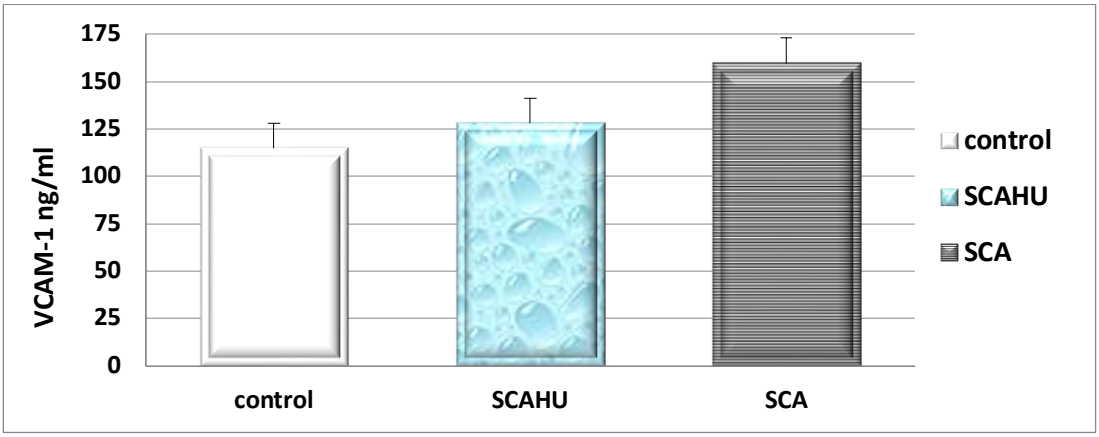

Figure 2: Comparison of plasma levels of VCAM-1 in control, SCA, and SCAHU groups.

In Figure 2, the data shows that there were no significant differences in the level of the plasma VCAM-1 between the three groups as p-value= 0.418 and the SCA group showed the highest level of VCAM-1 concentration. Data were expressed as mean ± SEM. Significance between groups was made using a nonparametric test (Kruskal-Wallis test).

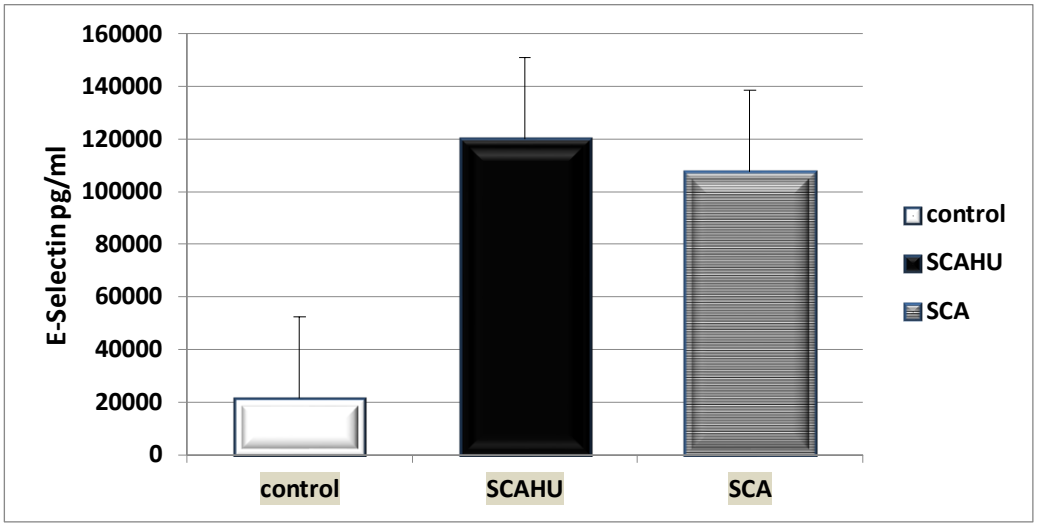

Figure 3: Comparison of plasma levels of E-Selectin in control, SCA, and SCAHU groups.

In Figure 3, the data shows that SCAHU recorded the highest level of E-Selectin compared to control and SCA, and there was a significant increase in the SCAHU group comparing to the control group as P value = 0.0002, also there is a significant increase in SCA group comparing to control group as P value = 0.0005. However, there was no significant difference between the SCAHU group and the SCA group detected as (P-value = 0.185). Data were expressed as mean ± SEM. Significance between groups was made using a nonparametric test (Mann-Whitney U test) between two groups and (Kruskal-Wallis test) between three groups.

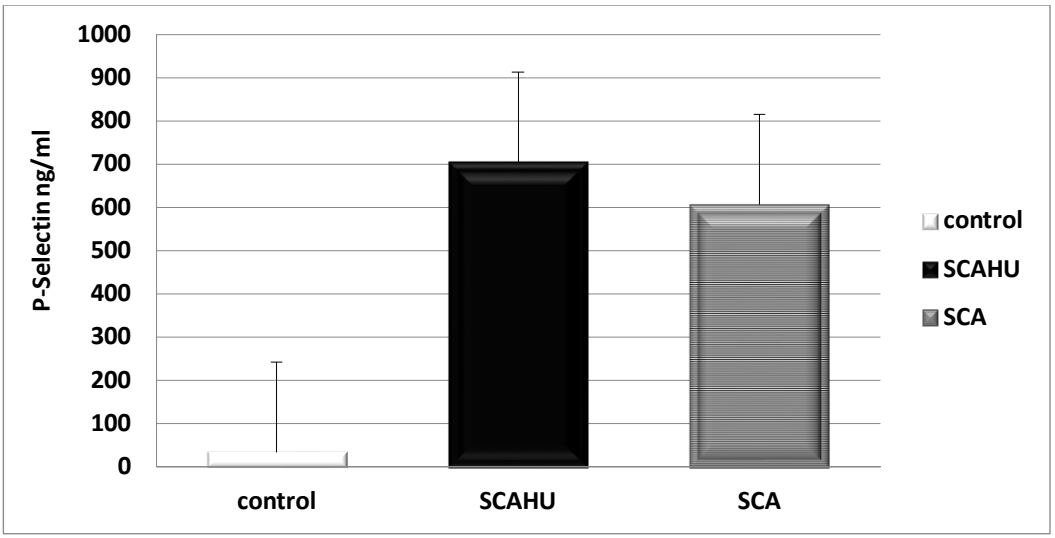

Figure 4: Comparison of plasma levels of P-Selectin in control, SCA, and SCAHU groups.

In Figure 4, the data shows that SCAHU had the highest concentration of P-Selectin compared to control and SCA. and there was a significant increase in the SCAHU group comparing to the control group as P value = 0.0002, also there is a significant increase in SCA group comparing

to control group as P value = 0.0001. However, there was no significant difference between the SCAHU group and the SCA group detected as (P-value =0.159). Data were expressed as mean ± SEM. Significance between groups was made using a nonparametric test (Mann-Whitney U test) between two groups and (Kruskal-Wallis test) between three groups.

5 Discussion

SCA is a genetic disease passed to children from their parents; patients with sickle cell report a poorer quality of life when compared to the population in general and other chronic non-communicable diseases [14]. It is the most common form of hemoglobinopathy worldwide [15]. A prevalence of 24 per 10,000 has been noted in Saudi children over 5 years old [16]. Chronic inflammation, recurrent ischemic reperfusion injury, and excessive levels of cell-free hemoglobin catalyzing oxidative reactions are causative of oxidative stress [17]. In addition, cell membrane peroxidation increases endothelial toxicity causing an upregulation of adhesion molecules which contribute to vaso-occlusion [18]. The resultant phenotypic expression of inflammation, vaso-occlusion, and microvascular injury to the organs occurs through a complex chain of cellular reactions [19].

The activation of endothelia is a significant factor in the cause of SCA; this can be seen in human blood cell cultures where sickle cells adhere to the endothelia. It has been shown that sickle deformed reticulocytes and RBC abnormally adhere to the vascular endothelium [20]. WBC also adheres to the endothelium forming hetero-cellular aggregates, adding to the small and large vessel occlusion [21]. The binding of leucocytes, reticulocytes, and sickle cells to the endothelial membrane was enhanced by the expression of endothelial adhesion molecules during endothelial activation caused cytokine stimulation [22]. These endothelial adhesion molecules are expressed in the blood at increased levels in sickle cell patients [23].

The up-regulation of the expression of adhesion molecules may be important in VOC development. During an infection episode, there are increased activated leukocyte numbers that express adhesion molecules at higher than baseline levels, which attach to the vascular endothelium and aid vessel occlusion. Many studies have shown that the most common predisposing factor for sickle cell crisis is infection [24]. Human and animal data indicate that the adhesion of leukocytes to other blood cells and the vascular endothelium is fundamental in the pathogenesis of vaso-occlusion in SCA [25].

Leukocytes have been shown to play a pivotal role in SCA pathophysiology. An increased leukocyte count in SCA has been linked to VOC, acute chest syndrome, and mortality [26]. In sickle cell disease (SCD), hypoxia regeneration injury results in enhanced leukocyte rolling [27], an activity that is regulated by L- and P-selectins [28]. After leukocyte rolling, there is upregulation of endothelial adhesion molecules, this causes cellular adhesion and interactions between leucocytes and endothelia. Integrins and other downstream molecules such as the immunoglobulin superfamily coordinate the firm leucocyte adhesion [29], which results in sickle red cell adherence (HbSS), which can further impair microcirculation blood flow [30].

In this study, adhesion molecule expression (VCAM-1, ICAM-1, P-selectin, and E-selectin) was measured in patients who receive no HU treatment (SCA), in patients receiving HU treatment (SCAHU),

and in the control subjects. Our study showed a significant increase in circulating levels of P-selectin and E-selectin in SCA patients compared with the control subjects. Also, there was a significant difference between the SCAHU group and the control group, indicating a higher level of E-selectin and P-selectin in SCAHU. However, there was no significant difference in the levels of E-selectin and P-selectin between the SCAHU and SCA groups. In addition, there were no significant differences in levels of VCAM-1, ICAM-1 in the plasma between SCA and SCAHU groups. Whereases, SCAHU group has a lower level of VCAM-1and ICAM-1 than SCA.

There is much evidence indicating that selectins are essential in sickle cell disease pathogenesis [31]. In the study conducted by Frenette et al. (2000) using homozygous sickle mice, both E- and P-selectin mediated the sickle erythrocytes adhesion of TNF-α stimulated venules that are compatible with our findings [32]. A murine model deficient in expression of P- and E-selectin did not develop leucocyte-mediated vaso-occlusion [33]. E-selectin is responsible for leucocytes rolling and the initial adherence of leucocyte-endothelia [29]. P-selectins role occurs at a later VOC stage, it helps to trap HbSS RBC to the adherent leucocytes [31]. *In vitro* HbSS cells incubated with mononuclear leucocytes resulted in leukocyte activation and follow by adhesion to human endothelial umbilical vein cells [34].

In another study, it was shown that blocking a P-selectin monoclonal antibody significantly reduced the leukocytes adhesion in cremaster venules of SCA transgenic mouse models. In this SCA animal model, an E-selectin-specific mAb was an ineffective treatment [35]. In another study, it was reported that patients with SCD had significantly increased soluble sE-selectin compared to the control subjects [36]. Studies using cultured endothelial and/or isolated cells also showed that P-selectin is involved in carrying out the adhesion of non-sickled and sickled RBCs derived from SCA patients to either monolayers of thrombin-stimulated or untreated human umbilical vein endothelial cells, as the adhesion response was reduced by anti-P-selectin mAbs [37]. Endothelial cells isolated from the circulation of patients with SCA showed increased P- and E-selectin expression [38]. Overall, these results support the idea that the activation of endothelial cells is associated with SCD and this activation caused increased E- and P-selectin expression, which contributed to circulation leukocyte and erythrocyte adhesion [39].

Another study was shown that endothelial cells exposed to sickle cells led to increased E-selectin expression. This shows that expressed E-selectin has a significant role in the pathophysiology of sickle-related complications. The E-selectin expression reduction may be useful for treating SCA. These results agree that sE- and sP-selectin are endothelial surface activation markers and they can be disease severity indicators [40].

On the other hand, there are typically low levels of ICAM-1 and VCAM-1 expression on the endothelial cells' surface. The expression can be induced by different types of biological stimuli, usually being inflammatory cytokines, including TNFα [41]. It has also been found that the expression of VCAM-1 and ICAM-1 is closely related to disease activity in many inflammatory processes, such as VOC [42]. In this study, we found that the levels of ICAM-1 and VCAM-1 in the circulation of SCA patients were increased when compared to the controls, but these results did not reach significant levels, and this is somewhat consistent with previous studies and may require studying a larger group of patients to fully understand their role.

Studies have shown there was an increased level of soluble ICAM-1 and soluble VCAM-1 in sickle cell anemia patients [23]. This is supported by other studies that have shown a significant increase in VCAM-1 in the plasma of sickle cell anemia patients, and it was also significantly increased in severe cases of SCA [43, 44].

Endothelial damage and activation levels can be correlated to the expression of soluble adhesion molecules including selectins, sVCAM-1, and sICAM-1. This agrees with studies that have shown endothelial adhesion molecule expression is increased in sickle cell patients [41, 45]. The abnormal endothelial activation is shown in this increase and it occurs even in a steady-state [46]. The disease severity is associated with these changes and also with complications associated with the disease, including pulmonary hypertension [41]. Several studies show that levels of soluble ICAM-1 and soluble VCAM-1 are increased in sickle cell anemia patients [47, 48].

ICAM-1 and VCAM-1 are among the most important inflammatory factors and play a major role in sickle cell anemia. P- and E-selectins are useful as markers for SCA management, as they are endothelial surface activation indicators and can be correlated with the severity of the disease. Unfortunately, there are not sufficient studies into the effects of P and E-selectin, and therefore further research is required.

In this study, there were no significant differences in levels of VCAM-1, ICAM-1, P- and E-selectin in the plasma between SCA and SCAHU groups. In [49], HU treatment was associated with significantly lower levels of red cell adhesion that were similar to those of control red cells. Previous reports showed that HU treatment reduces VCAM-1 and ICAM-1 expression in patients with SCA [23,50] and decreases P- and E-selectin in the plasma of sickle cell patients [45,51,52].

6 Conclusion

The increased expression of P-selectin and E-selectin influences patients to SCD severe manifestations, and the increased expression of adhesion molecules could be used as an indicator for sickle cell crises development. The understanding of the mechanism of increased adhesion molecules will help for developing improvements in the therapies available to treat SCA. Our findings show that ICAM-1 and VCAM1 were increased in all patient groups compared to the control group, but this change did not reach significant levels. However, adhesion molecules tended to fluctuate depending on external factors and the clinical conditions of the patient. Also, we did not find any evidence of a significant effect of HU on the patient groups. However, ICAM-1, VCAM-1 levels were decreased in SCAHU patients. Further research is required with a larger group of participants to fully sightsee the role of adhesion molecules with HU to develop a targeted approach to counteract the disease manifestations.

7 Availability of Data and Material

Data can be made available by contacting the corresponding author by email.

8 Acknowledgments

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9 Ethics Statement

This work has been approved by the Biomedical Research Ethics Committee, Ministry of Higher Education, King Abdulaziz University, Faculty of Medicine (Reference No 243-19).

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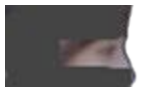

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