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The effect of hydroxyurea on the expression of white blood cell adhesion molecules in patients with sickle cell disease

SCD:

-Autosomal recessive

-Serious chronic hemolytic anemia.

-Replacement of glutamic acid with valine in the sixth amino acid position

Sickle cells:

-Sickle cells are tougher than the normal cells and are less flexible

-The toughness and deformity lead to chronic hemolysis, loss of plenic function, recurrent infections, and vaso-occlusive crisis (VOC).

HbS polymerizaon:

-Damages RBC membranes

-Disrupts cellular cation homeostasis

-That results in loss of potassium, water, and dehydration of cells

-Finally, erythrocytes become irreversibly sickle.

VOC:

-The VOC is the most important cause of morbidity and mortality in patients with SCD.

- It is a complex process in which different factors are involved, including erythroid and leukocyte adhesion molecules, inflammation, endothelial damage, activation of platelets and coagulation process, and the reduction of nitric oxide.

WBC:

-Leukocytes or white blood cells (WBC) play an important role in incidence and exacerbation of VOC

-WBCs are larger than erythrocytes and less flexible

- They can adhere to the endothelium, especially in small vessels, and disrupt RBC and WBC movement, and consequently,increase risk of VOC

WBC:

- WBCs play a core role in pathophysiology of SCD.
- There is a direct correlation between leukocytosis and exacerbation of the disease.
- Leukocytosis as a risk factor increases the incidence of crises, such as acute chest syndrome, stroke, and early death.
-The increase is mainly related to the neutrophils in patients

WBC:

-Considering the increase in percentage of neutrophils in this study, it seems that neutrophils play an important role in pathogenesis of SCD, as the activity of these cells increase in the course of the disease besides their count

Admission molecules:
- adhesion of leukocytes to the endothelium and other cells is mediated by adhesion molecules, such as M2(CD11b/CD18),
L2(CD11a/CD18), CD62L, CD162, and CD64, binding to ICAM-1, VCAM-1, and ICAM-4 ligands on the endothelial and other cells.
-Studies show that the expression of leukocyte adhesion molecules increased in patients with SCD compared to that in normal participants.
- Neutrophils in patients with SCD had greater affinity for binding to endothelial and fibronectin

Adhision molecules:

-Expression of adhesion molecules on leukocytes showed that the expression of M2 (CD11b/CD18) on neutrophils and CD62L on neutrophils and lymphocytes significantly increased in patients with SCD.

-Improvement of clinical signs by HU before the increase in hemoglobin F was probably due to the reduction in expression of adhesion molecules by HU.

Adhision molecules:

-Expression of CD11b on neutrophils and CD11a on lymphocytes was higher in patients with SCD than that in normal people.

-Leukocytes adhere to vascular wall through binding of integrin molecule subunits, such as CD11a, CD11b, CD18, and CD62L (L-selectin), to adhesion molecules of vessels happens through ICAM-1, Eselectin, P-selectin, and VCAM-1.

Inflamation

-The expression of these adhesion molecules on the endothelium of vessels probably happens after a vascular inflammation.

-Numerous inflammatory markers, such as TNFα, C-reactive protein, IL-8, and IL-1β, were reported to be in blood circulation of patients with SCD.

-The adhesion of active neutrophils to the wall of vascular endothelium cultured in the presence of inflammtory markers

Hydroxyurea indications (clinical and preventive) : All children with sickle cell disease (SS or S/b0 thal) age 9 months or older regardless of disease severity.1 Clinical symptoms: dactylitis or pain crises, acute chest syndrome, hemolytic crisis requiring transfusion. Conditional transcranial Doppler imaging. Parent preference for primary stroke prevention after transcranial Doppler normalization with chronic transfusion.

- Parent preference for management of silent cerebral infarct (offer hydroxyurea vs chronic red cell transfusions). Secondary stroke prevention to augment goals of chronic red cell transfusions in patients with significant anemic, progressive vasculopathy with chronic red cell transfusions.

- Patients where chronic red cell transfusions is not an option: lack of intravenous access, rare blood types, alloimmunization, or severe iron overload. Organ protection and modification of disease complications in adults.

HU:

1-Today, hydroxyurea (HU) is one of best drugs used for treatment of with SCD and has reduced mortality of these patients by 40%.

2-HU disrupts the cellular cycle through inhibition of ribonucleotide reductase.

3-However, molecular target of HU and the way of improving its clinical symptoms are not known completely

HU:

-It was first supposed that HU increased hemoglobin F, but then, it was observed that the clinical improvement happened before the increased hemoglobin F.

- It was proposed that HU probably worked through other mechanisms, such as reducing leukocyte and erythroid adhesion to the endothelium and components of extracellular matrix

HU:

-The increase is mainly related to the neutrophils in patients, as the percentage of neutrophils in the peripheral blood decreased
considerably after using HU.

- HU decreased neutrophil count, and subsequently, leukocytosis decreased, and clinical signs were improved prior to a
change in the level of hemoglobin F.

- Treatment with HU significantly decreases the expression of adhesion molecules on leukocytes and also neutrophil count

Methods: patients were divided into two groups

-21 patients who suffered sickle cell disease and received hydroxyurea,
-21 patients who suffered sickle cell disease but did not receive hydroxy urea
.-control group comprised 21 healthy people.
-A blood samples was drawn from all participants, and a CBC test was performed to determine WBC
- After Lysis of red blood cells by lysine, blood samples were stained with four types of monoclonal antibodies against surface antigens of CD11a, CD11b, CD18, and CD62L and analyzed using flow cytometry.
- Finally, the expressions of these markers on leukocytes were recorded as percent values.
- The indexes used for the three groups were compared to one another using SPSS software, ANOVA, and Tukey test

Results:

- WBC count in patients was significantly higher than that in the control group.
-Affter using hydroxyurea, it decreased significantly in patients (P- value≤ 0.05).
- Percentage of neutrophils was high in patients, but it decreased significantly after using hydroxyurea.
-The analysis by flow cytometry showed that the expression of CD11a, CD11b, CD18, and CD62L markers on leukocytes increased in
patients compared to that in the control group.
-The expression of these markers in patients with sickle cell disease significantly decreased after using hydroxyurea (P-value≤ 0.05)

Table 1: The comparison of mean and standard error mean (SEM) of white blood cell numbers among healthy individuals (Control group), sickle cell disease patients (SCD group) and sickle cell disease patients using hydroxyurea (SCD-HU group).

P-value	SCD-HU group (Mean±SEM)	SCD group (Mean±SEM)	Control group (Mean±SEM)	white blood cell (No.)
0.018	8177 ± 800	11410 ± 1400	7490 ±280	WBC

Table 2: The comparison of mean and standard error mean (SEM) of leukocyte subsets among healthy individuals (Control group), sickle cell disease patients (SCD group) and sickle cell disease patients using hydroxyurea (SCD-HU group) according to percent (%).

P- vlaue	SCD-HU group (Mean±SEM)	SCD group (Mean±SEM)	Control group (Mean±SEM)	Leukocyte subsets
0.005	60.14 ± 1.86	70.19 ± 2.50	65.86 ± 2.08	Neutrophils
0.605	31.15 ± 2.35	30.47 ± 2.06	33.48 ± 2.12	Lymphocytes
0.068	2.250 ± 0.393	2.850 ± 0.254	1.875 ± 0.239	Monocytes
0.225	1.692 ± 0.286	2.105 ± 0.215	1.545 ± 0.207	Eusinophils

Table 3: The comparison of mean (M) and standard error mean (SEM) of leukocyte expression of different markers among healthy individuals (Control group), sickle cell disease patients (SCD group) and sickle cell disease patients using hydroxyurea (SCD-HU group) according to percent (%).

P- vlaue	SCD-HU group (Mean±SEM)	SCD group (Mean±SEM)	Control group (Mean±SEM)	Leukocyte expression of markers
0.023	25.14 ± 1.91	33.78 ± 2.65	29.38 ± 1.84	CD11a
0.003	21.96 ± 2.41	32.27 ± 2.47	24.63 ± 1.24	CD11b
0.008	42.31 ± 1.92	51.10 ± 2.42	44.54 ± 1.51	CD18
0.018	23.06 ± 1.33	27.55 ± 2.07	21.49 ± 0.967	CD62L

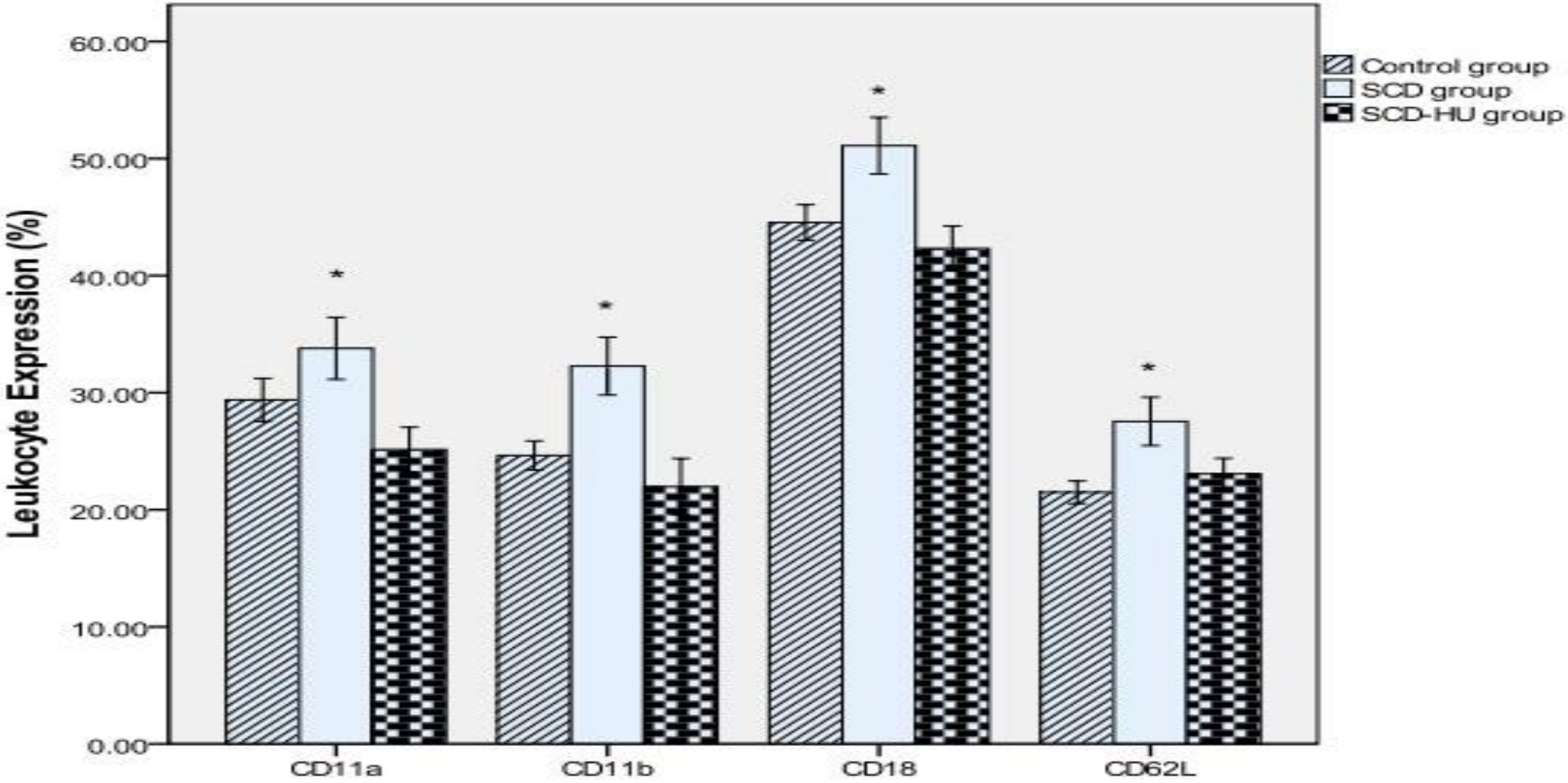

Figure 1: Leukocyte expression of different markers (Adhesion molecules) among Control group, sickle cell disease patients (SCD group) and sickle cell disease patients using hydroxyurea (SCD-HU group). Note: The expression of these markers increased in the patients but decreased after using hydroxyurea (*P*-value≤0.05)

Conclusion:

-Pharmacological techniques that can reduce adhesion of leukocytes to the vascular wall may be an important strategy in prevention ofVOC.

- In this regard, the reduction of adhesion molecules were reported in patients with SCD receiving HU.

Thanks for your attention