CXC chemokines CXCL1, CXCL9, CXCL10 and CXCL12 are associated with sickle cell disease and carriers: a study of patients from the southeast region of Iran

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INTRODUCTION
Sickle cell disease (SCD): a recessively inherited chronic hemolytic anemia
abnormal haemoglobin Hbss
Caused by a single nucleotide substitution in the β globin gene on chromosome 11

Hemoglobin S (most common): GTG → GAG results in substitution of valine (hydrophobic) for glutamate (hydrophilic)

Mutant hemoglobin polymerizes under low oxygen conditions and form bundles that distort red cells into the classic sickle shape
Figure 3-13 Biological Science, 2/e
Pathophysiology

**Hemoglobin S**

- Deoxygenation
- Polymerization of hemoglobin
- Sickling of red cells
- Endothelial damage/activation
- RBC and leukocyte adhesion to endothelium, vasoconstriction
- Vascular occlusion, organ ischemia and end-organ damage
Chemokines
Classification of chemokines

C chemokines

CC chemokines

CXC chemokines

CX3C chemokines

peptide chain

disulphide bridge

hydrophobe domain

mucine-like domain

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## Chemokine producer cells

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<th>CXC chemokine</th>
<th>CC chemokine</th>
<th>CX3C chemokine</th>
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<td>monocyte</td>
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<td>lymphocyte</td>
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* SCD exhibits a form of mild chronic low-grade inflammatory state
* In SCD, endothelial activation is associated with circulating leukocytosis and monocytosis along with increased soluble vascular cell adhesion molecules
* Inflammation and angiogenesis are both involved in the pathophysiology of SCD
* Various functions are described for chemokines varying from involvement in defensive actions, reconstruction of damaged tissues to regulation of angiogenesis anangiosis
Evidence demonstrated that plasma levels of several chemokines and cytokines including tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), IL-6, IL-8, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), and interferon γ (IFN-γ) are elevated in SCD patients.

CXCL1 (Growth related oncogene-alpha) is a well-known member of the CXC chemokine family. CXCL1 is involved in the pathogenesis of atherosclerosis, angiogenesis, and inflammation.
their pro-inflammatory properties, CXCL9 and CXCL10 are anti-angiogenic members of the CXC chemokine subfamily and have been demonstrated to act as recruiters of activated T-helper 1 (Th1) and natural killer (NK) cells. CXCL10 is also involved in lymphocyte recruitment in inflammatory related disorders such as autoimmune thyroid disorders and demyelinating neuropathies. Moreover, elevated CXCL10 was observed in type 1 diabetic patients.

The Stromal-Derived Factor 1 (SDF-1) which in recent nomenclature is called CXCL12 also fits into the CXC group. CXCL12 has also been reported as key mediator for angiogenesis and inflammation. Previous studies evidenced elevated CXCL12 in SCD patients. CXCL12 levels were also significantly higher in SCD patients suffering from pulmonary hypertension in comparison to patients without this complication.
We aimed and designed the present study to examine whether the serum levels of CXC chemokines CXCL1 and CXCL12 (as proangiogenesis) and CXCL9 and CXCL10 (as angiostasis) are involved in pathogenesis of SCD.
MATERIALS AND METHODS
This cross-sectional study was conducted at the Kerman Special Disease Center and Rafsanjan Molecular Medicine Research Center. 77 children with SCD and 70 controls were enrolled in the study. Peripheral blood samples were collected from patients in 5 mL EDTA pre-coated tubes.

Patients having a history of major thalassemia, inherited bone marrow failures, and leukemia, history of other inflammatory states such as type-1 diabetes and immune system related disorders.
blood sample was taken from each patient to determine the chemokine levels and clinical parameters.

urine was collected for determination of albuminuria. Demographic and medical history of patients including age, gender and duration of using hydroxyurea (HU) were obtained from medical records.

The protocol was approved by the local Ethics Committee of Rafsanjani University of Medical Sciences and an informed consent form was filled out by parents of SCD children and controls.
The serum levels of CXCL1, CXCL9, CXCL10, and CXCL12 were measured by ELISA (R&D systems, UK) in patients and healthy controls following sample collection. Assays were performed according to the manufacturer’s guidelines.

- Measurement of chemokines

ALT and AST
WBCs (white blood cells)
serum ferritin level
microalbuminuria

- Measurement of clinical parameters
Statistical analysis

* Statistical analysis was carried out using SPSS version 15.
* The difference between two groups was evaluated using Student's t-test. Pearson’s correlation test was also applied for statistical analysis of correlations between variables.
* P values of less than 0.05 were considered significant.
RESULTS
Seventy seven SCD patients and 70 control subjects were enrolled in the study. The mean ages of the studied groups were 11.3 ± 0.9 and 13 ± 2 years in SCD patients and controls, respectively. Almost all of the SS patients had anemia with a hemoglobin level of approximately 8.59 ± 1.41 g/dL. The mean duration of HU treatment of the patients was 3.27 ± 3.05 years and 54 patients were using HU. There was no significant change in the level of CXC chemokines in SCD patients under treatment with HU and those not under treatment (p > 0.05; Figure 1).
Present study demonstrated that the mean circulating levels of CXCL1 in SCD patients and control was 223.32 ± 20.5 pg/mL and 141.27 ± 18.1 pg/mL, respectively.

The mean concentration of circulating CXCL9 in SCD patients and control was 159.76 ± 12 pg/mL and 212.57 ± 12.6 pg/mL, respectively.

Findings of the investigation indicated that circulating CXCL10 in SCD patients and controls was 177.56 ± 12.6 and 180.75 ± 18.4, respectively.

Circulating level of CXCL12 in SCD patients and controls was 82.77 ± 4.9 pg/mL and 46.57 ± 8.1 pg/mL, respectively.
Analysis of data by Pearson’s correlation test, revealed a significant correlation between the level of CXCL1 with HbS ($r = 0.474$, $p = 0.04$), AST ($r = 0.692$, $p = 0.001$), and ALT ($r = 0.016$, $p = 0.652$) but not other investigated chemokines.

These results demonstrated that CXCL1, CXCL9, CXCL10, and CXCL12 were increased in SCD patients suffering from pain crisis, however, the difference was not significant. Finally, these results demonstrated that there was no significant difference between micro-albuminuria, serum ferritin, and WBC count in SCD patients suffering from pain crisis as compared to patients without pain crisis.
DISCUSSION
SCD is a chronic disease state which affects multiple organs.

The status of patients is manifested by leukocytosis accompanied by enhanced circulatory levels of proinflammatory chemokines and cytokines.

In this study we enrolled 77 patients with SCD and 70 control subjects. Study subjects were matched for age, gender, and more importantly, ethnicity and shared a geographical region in the southeastern part of Iran.

This is the first study to address a role for these CXC (CXCL1, CXCL9, CXCL10, and CXCL12) chemokines in SCD. We have chosen a set of CXC chemokines involved in inflammatory, angiogenesis, and angiostasis processes to explore whether they play a role in SCD.

Treatment with HU has been documented that induces numerous proinflammatory including, IL1α, IL1β, IL 6, IL 8, CCL2, CCL5, CCL20, and CCL8. In contrast to the evidences on the regulatory effects of HU on inflammatory cytokines, in our study HU has not affected the expression of the chemokines CXCL1, CXCL9, CXCL10 and CXCL12.
More recent evidence has indicated that several proinflammatory cytokines including TNF-α, IL-1β, IL-6, and IFN-γ and chemokines such as IL-8, CCL2, and CCL4 are elevated during a painful crisis of SCD.

The pain crisis complications observed in SCD are most often associated with organ dysfunction and injuries, including hypoxia, and also the release of hypoxia related mediators such as reactive oxygen species, hypoxia induced factors (HIF-1, HIF-2, etc.). These mediators, that up-regulate proinflammatory chemokines, are well evidenced.

Thus, it is possible to conclude that the increased level of these chemokines is due to the above facts. Therefore, the release of proinflammatory as well as angiogenic CXC chemokines in SCD patients is probably a response of injured tissues to injury and recruitment of competent cells to overcome the insult and injury mediated destructive damage.

Therefore, this is consistent with our current report regarding increased levels of CXCL1, CXCL9, CXCL10, and CXCL12 (however, it did not differ significantly) our previous reports on increased levels of these chemokines in different inflammatory states including type 1 diabetes, multiple sclerosis, premature birth [and cell systems], SCD may possibly follow this pattern of chemokine expression.
thankyou
Using the t-test, we found that the circulating levels of the studied CXC chemokines CXCL1 and CXCL12 were significantly increased in SCD patients in comparison to control subjects (p = 0.002, p < 0.001; Figure 2). Inverse results also showed that the circulating levels of CXCL9 and CXCL10 were decreased in SCD patients in comparison to control subjects (p = 0.001, p > 0.05; Figure 2).
Conclusions

According to the results of this study it can probably be concluded that the balance between angiogenesis/angiostasis CXC chemokines is an important predictive factor for initiation of complications in SCD patients. The elevated level of pro-inflammatory CXC chemokines may also be related to inflammatory responses associated with SCD complication.