Published Online: 2024 November 5 **Research Article**

Association of Clinical and Paraclinical Factors with Pain Crisis Severity in Sickle-Thalassemia Patients in Iran

Vahid Mohammad Karimi [1], Mehdi Dehghani [2], Maryam Rast [3], Mahya Amini [1,*]

[1] Hematology Research Center, Shiraz University of Medical Science, Shiraz, Iran
[2] Department of Hematology and Medical Oncology, Hematology Research Center, Namazi Hospital, Shiraz University of Medical Science, Shiraz, Iran
[3] Shiraz University of Medical Science, Namazi Hospital, Shiraz, Iran

[*] ***Corresponding Author:*** Hematology Research Center, Shiraz University of Medical Science, Shiraz, Iran. Email: aminimahya2020@gmail.com

Received: 14 April, 2024; **Revised:** 17 September, 2024; **Accepted:** 29 September, 2024

Abstract

Objectives: This study aimed to evaluate the effects of several factors, including hospitalization frequency, inflammatory and hemolytic markers, and the numbers of blood transfusions and crises, on the severity of pain crises in sickle-thalassemia patients.

Methods: Of all patients visiting hematologists in university-affiliated hospitals and clinics, only 75 sickle cell disease (SCD) and sickle-thalassemia patients met the inclusion criteria for this study. Disease severity was measured based on pain crises per year and laboratory markers, including hemolytic and inflammatory indicators, outpatient or inpatient status, the number of hospital admissions, and the number of blood units transfused over one year.

Results: In sickle cell patients, a significant correlation was observed between hospital admission and WBC (0.01), ALP (0.001), MCHC (0.001), ferritin (0.021), and ESR (0.006). Additionally, pain crises were positively correlated with transferrin saturation (TS) (+0.28) and the number of transfused blood units (+0.49) and negatively correlated with Hb (-0.30) and total iron binding capacity (TIBC) (-0.23). Blood unit transfusions showed a positive correlation with serum iron (+0.44), RDW (+0.36), ferritin (+0.39), pain crises (+0.49), and TS (+0.49), along with a negative correlation with Hb (-0.75) and MCHC (-0.33). When categorizing pain crises into two groups, patients experiencing more frequent crises generally exhibited lower hemoglobin levels and a higher number of blood units transfused. Hemoglobin also showed a significant negative correlation with lactate dehydrogenase (LDH) level in both groups: -0.604 ($P = 0.005$) in patients with ≥ 3 crises per year and -0.368 ($P = 0.006$) in patients with < 3 crises per year. Patients with hemoglobin levels of 7.5 or higher tended to maintain an LDH level below 1000. Among patients referred with pain crises, hemoglobin was negatively correlated with hemolytic markers such as LDH (-0.41) and retic (-0.29).

Conclusions: Hemoglobin and LDH can serve as follow-up markers, as they are routinely measured in all sickle cell patients and, in this study, hemoglobin levels of 7.5 or higher and LDH levels below 1000 were associated with fewer and less severe pain crises. Regular monitoring of hemolytic markers may help in pain crisis prevention, as this study found that hemolytic crises frequently coincided with pain crises. Further research is needed to provide conclusive evidence in this area.

Keywords: Sickle-Thalassemia, Clinical and Paraclinial, Pain Crises

1. Background

Sickle cell disease (SCD) is an autosomal recessive hemoglobin disorder, with approximately 300000 new cases diagnosed globally each year (1, 2). Sickle cell disease is prevalent among Iranians, particularly those in the southern regions, where the prevalence of sickle cell trait and sickle cell anemia is estimated to be around 1.43% and 0.1%, respectively (3, 4). In this condition, glutamic acid is replaced by valine at the sixth position of the hemoglobin beta chain, leading to the formation of hemoglobin S. Affected individuals may be either homozygous or compound heterozygous, resulting in different types of SCD, including sickle beta-thalassemia, each with a broad spectrum of clinical manifestations (5-7).

The primary event in sickling involves the polymerization of red blood cells, often triggered by factors such as hypoxia and infections. As a result, defective RBCs become trapped in small blood vessels, leading to acute crises, chronic hemolysis, tissue ischemia, disabilities, and, in severe cases, premature death (5, 8). Other factors, including interactions among multiple adhesion molecules and receptors on red blood cells, white blood cells, platelets, and endothelial cells, further complicate this process (9-11).

Laboratory parameters play a significant role in assessing SCD's clinical manifestations. Indirect markers of red blood cell lifespan, such as plasma hemoglobin, serum bilirubin, reticulocyte count, lactate dehydrogenase (LDH), and alanine aminotransferase (ALT), are not only diagnostic but also prognostic indicators (12-14).

Most individuals with SCD face lifelong disabilities due to both acute crises and chronic damage to various organs (15, 16). Among factors affecting survival, laboratory parameters are especially impactful; for example, hemoglobin genotype significantly influences disease severity. Patients with homozygous hemoglobin S (HbSS) have lower survival rates compared to those with HbSB* (17, 18).

Hemoglobinopathies, including sickle cell anemia and sickle-thalassemia traits, are prevalent in Iran, imposing a substantial burden on healthcare systems. Optimal management requires addressing serious complications such as splenic infarction, osteomyelitis, transfusion-related issues like iron overload, and severe transfusion reactions. These disorders also present significant challenges for patients, who frequently experience recurrent admissions and develop analgesic dependence to manage pain. Thus, the ability to diagnose attacks preemptively through laboratory markers could help mitigate the many adverse effects and complications associated with delayed detection of sickle cell pain and hemolytic crises.

2. Objectives

Given the potential association between hemolysis markers and disease severity, we examined correlations between hemolytic factors (AST, ALT, bilirubin, Hb, PLT, reticulocyte count) and inflammatory markers (ferritin, ESR, CRP, WBC, PLT). Additional factors related to disease severity were also investigated, including the number of pain crises, blood transfusions, inpatient or outpatient follow-ups, and hospital admissions for patients with both sickle cell and sickle-thalassemia phenotypes.

3. Methods

This cross-sectional study included all patients with sickle cell anemia and sickle-thalassemia traits receiving inpatient or outpatient follow-up care at Namazi and Amir Hospitals and Amir Clinic under hematologist supervision over a one-year period, from January 2018 to January 2019. Inclusion criteria were ages 18 - 70, absence of other underlying diseases, and complete medical records.

After obtaining consent, demographic data (age and sex) and disease severity factors (pain crises per year, outpatient or inpatient visits, hospital admissions, and number of blood transfusions received) were recorded. Blood samples were collected to measure complete blood count, reticulocyte count, liver function markers (AST, ALT, bilirubin, and ALP), biochemical markers (BUN and Cr), LDH, serum iron, total iron binding capacity (TIBC), and ferritin levels.

Data were analyzed using SPSS 25 with various statistical tests. For quantitative data, means and standard deviations were calculated, while frequency and percentages were used for qualitative data. An independent *t*-test was applied for normally distributed quantitative data compared with binary qualitative variables. For non-parametric data, the Mann-Whitney test was used. Additionally, the Kruskal-Wallis test was performed for quantitative variables compared with multiple qualitative categories. Finally, correlations between two quantitative variables were assessed with the Spearman correlation coefficient. A P-value of less than 0.05 was considered statistically significant.

4. Results

Of the patients enrolled in the study, 48% were female, and 52% were male, with a mean age of 33.56 ± 1.27. Equal numbers of sickle cell and sickle-thalassemia patients participated, with proportions of 50.7% and 49.3%, respectively. Among participants, 65.3% had a history of hospital admissions, while 34.7% did not. Additionally, 52% of patients had received blood units at some point in their disease course, while 48% had no transfusion history (Table 1).

The study aimed to assess the relevance of certain laboratory parameters in sickle cell and sickle-thalassemia patients in relation to the outlined severity indices. No association was found between sex and

Table 1. Demographic Characteristics of Patients with Sickle Cell Disease and Sickle Thalassemia

Parameters	SCD	Sickle Thalassemia
Number	38	37
Age (y)	34.42 ± 11.8	32.67 ± 10.37
Gender ratio	22 females/16 males	22 females/16 males
Hospital admission	24	25
Transfusion history	18	21
Pain crises	38	37

Abbreviation: SCD, Sickle Cell Disease.

other factors. However, with increasing age, MCHC increased (+0.24), while ESR (-0.29) and CRP (-0.24) decreased, as indicated by Spearman's rho test. Additionally, inverse correlations were observed between pain crises and both hemoglobin level (-0.30) and TIBC (-0.23), while positive correlations were found with transferrin saturation (TS) (+0.28) and the number of transfusions (+0.49), again based on Spearman's rho test.

A significant difference was observed between hospital admissions and Hb (0.001), MCHC (0.001), and RDW (0.012) in the independent *t*-test, while relationships were identified between hospital admissions and WBC (0.012), ALP (0.001), ferritin (0.021), and ESR (0.006) using the Mann-Whitney test. Further associations were found between blood unit transfusion and LDH (0.035), PLT (0.009), and ferritin (0.013) via the Mann-Whitney test. The number of blood units showed positive correlations with serum iron (+0.44), RDW (+0.36), ferritin (+0.39), crises (+0.49), and TS (+0.43), and negative correlations with Hb (-0.75) and MCHC (-0.33), as assessed by Spearman's rho test.

Next, patients with pain crises were categorized into two groups: Those with fewer than three annual events and those with three or more. In both groups, higher numbers of crises corresponded to lower mean hemoglobin levels. Furthermore, patients who received more blood units had lower hemoglobin levels, with the second group showing significantly lower mean hemoglobin levels overall. In the first group, the P-value was significant (0.003), while for patients experiencing three or more crises annually, the P-value was 0.223 (Table 2).

Given that LDH was significantly correlated with Hb (P-value: 0.001) and is a convenient and affordable test routinely used to assess the risk of hemolytic anemia, we conducted further analysis to explore the relationship between hemoglobin levels and LDH in two patient groups: Those with three or more pain crises per year and those with fewer than three episodes annually. A significant correlation was observed between hemoglobin levels and LDH in both groups, with P-values of 0.005 and 0.006, respectively. Additionally, Spearman's rho test revealed a negative correlation between hemoglobin and LDH in both groups (-0.604 and -0.368), with the correlation being more pronounced in patients experiencing three or more pain crises annually (Table 3).

Notably, we observed that except for patients with fewer than three crises per year who had received only one blood unit, other patients had hemoglobin levels of approximately 7.5 or lower. We thus established 7.5 as a reference threshold and evaluated its correlation with LDH. Results showed that patients with hemoglobin levels of 7.5 or higher had lower LDH values compared to those with hemoglobin levels below 7.5 (963 ± 103.04 vs. 1451.126 ± 279.71, respectively; Table 4).

Another noteworthy finding was that patients presenting with pain crises generally had lower hemoglobin levels. To investigate a possible concurrent occurrence of hemolytic crises with pain crises, we conducted a Spearman's rho correlation test between Hb levels and hemolytic markers, including LDH, reticulocyte count, AST, direct and indirect bilirubin, and ALT. A correlation was identified between Hb and specific hemolytic factors, such as LDH (-0.41) and reticulocyte count (-0.29), in Spearman's rho test (Table 5).

Conversely, we conducted the same analyses for patients with sickle-thalassemia but found no significant correlations in this group.

5. Discussion

Sickle cell disease is prevalent across regions including sub-Saharan Africa, the Mediterranean, the Middle East, and India (19). Vaso-occlusive crises and

Table 2. Comparison of Mean Hemoglobin Levels with the Number of Transfused Blood Units in Two Groups of Patients Experiencing Pain Crises: Those with ≥ 3 Crises Annually vs. Those with < 3 Crises Annually [a]

Number of Pain Crises	Crises < 3	P- Value	Crises ≥ 3	P-Value
1	8.61 ± 0.22, 8.60 (1.00)	0.003 [b]	6.8 ± 1.19, 7.10 (0.00)	0.223 [b]
2	7.61 ± 0.26, 7.85 (0.90)		6.75 ± 0.49, 7.10 (1.70)	
3	6.83 ± 0.60, 6.50 (2.00)		5.7 ± 1.1, 5.7 (0.00)	
4	6.25 ± 0.06, 6.20 (0.00)		5.05 ± 0.45, 5.05 (0.00)	

[a] Values are expressed as mean ± SD, median (IQR).

[b] Kruskal-Wallis test.

Table 3. Spearman Correlation Between Hemoglobin and Lactate Dehydrogenase in Patients with ≥ 3 Pain Crises Per Year Compared to Those with < 3 Crises Annually

Variable	Crises < 3	P-Value	Crises ≥ 3	P-Value
Hemoglobin	LDH	0.006 [a]	LDH	0.005 [a]
	-0.368		-0.604	

[a] Spearman's rho correlation test.

Table 4. Comparison of Mean Lactate Dehydrogenase Levels with Hemoglobin Levels in Sickle Cell Patients [a]

Variable	Hemoglobin		P-Value
	< 7.5	≥ 7.5	
LDH	1451.26 ± 279.71, 893.00 (1429.00)	963.12 ± 103.04, 755.00 (667.75)	0.023 [b]

Abbreviation: LDH, lactate dehydrogenase.

[a] Values are expressed as mean ± SD, median (IQR).

[b] Mann-Whitney U test.

chronic hemolysis are two primary clinical events in SCD, with ischemic pain from vaso-occlusion being the most common crisis, presenting in various clinical severities (20, 21).

Overall, we did not find any significant link between disease phenotypes and severity factors, such as the number of transfused blood units, pain crises, admissions, or other hemolytic parameters. Due to distinct phenotypes, patients present with diverse clinical manifestations; for example, individuals with HbSS and sickle B0-thalassemia are known to experience more severe courses than those with HbSC or HbSB+ phenotypes (22). Given that this study was retrospective and full Hb electrophoresis data was unavailable for all patients, we presumed that the sickle thalassemic patients likely had the sickle B0 genotype, thus sharing clinical features with SCD.

Our study identified a negative correlation between Hb and TIBC with the frequency of pain crises. A lower hemoglobin level, combined with low MCHC and high RDW, could potentially trigger pain crises due to reduced oxygen-carrying capacity and an increased HbS/HbA ratio. Previous studies suggest that an ideal hemoglobin level of 10 is optimal in homozygous HbS patients; however, achieving this goal poses challenges due to the risk of alloimmunization from frequent transfusions and their serious consequences (23-25). In this study, when categorizing patients by crisis frequency, we found that lower hemoglobin levels were associated with higher incidences of pain crises, aligning with previous findings. Patients with a mean Hb level of 9.8 did not experience any crises.

Total iron binding capacity also showed a notable effect in our study, exhibiting a negative correlation with the number of pain crises (-0.23) and a positive correlation with TS (+0.64). The average TIBC in sickle cell patients was lower than in healthy individuals, which may reflect iron overload due to increased absorption and chronic hemolysis (26). Higher TS is indicative of iron overload, which, due to its pro-

Table 5. Correlation of Hemoglobin with Hemolytic Factors: Negative Correlation Between Hemoglobin Levels and Both Lactate Dehydrogenase and Reticulocyte Count

Parameter	LDH	T.Bili	Indirect Bilirubin	Reticulocyte Count	AST	ALT
Hemoglobin	-0.409	+0.069	+0.115	-0.288	+0.041	-0.023
P-value [a]	0.001	0.56	0.33	0.013	0.72	0.84

Abbreviation: ALT, alanine aminotransferase.

[a] Spearman's rho correlation test.

inflammatory role and bone marrow toxicity, results in ineffective erythropoiesis and a lower hemoglobin level, ultimately contributing to more frequent pain crises (27-29). The observed negative correlation between pain crises and both Hb and TIBC, along with the positive correlations with TS and transfusion frequency, supports this understanding.

Patients admitted to the hospital exhibited lower hemoglobin levels (7.9 ± 1.90), reduced MCHC, and increased RDW, ferritin, ALP, WBC, and ESR, which supports the role of chronic hemolysis and inflammation, such as infections, as common causes of pain crises. These results align with previous studies indicating that markers like ferritin, WBC, and ESR can serve as inflammatory indicators predictive of pain crises or their severity (9, 30).

Additionally, the correlation found between blood transfusions and elevated ferritin and LDH levels is compelling, as these markers often increase in conditions such as iron overload, inflammation, and hemolysis. These conditions are thought to trigger or worsen crises by promoting inflammation and chronic hemolysis (30-32).

Our study further revealed that patients experiencing more frequent pain crises (≥ 3 annually) had lower hemoglobin levels than those with fewer than three episodes per year and required more frequent blood transfusions. Excluding patients who had fewer than three annual crises and received only one blood unit (with an average hemoglobin of 8.61 ± 0.22), other patients had an average maximum hemoglobin level around 7.5 (7.61 ± 0.26) (Table 2). A strong negative correlation was also observed between hemoglobin levels and LDH (Table 3). Furthermore, as previously noted, LDH levels were slightly below 1000 in patients whose hemoglobin was over 7.5 (Table 4). Thus, based on these findings, we suggest that a hemoglobin level of 7.5 or higher and an LDH below 1000 may indicate a favorable prognosis in this patient group. This conclusion supports the observed relationships between pain crises, mean hemoglobin level, LDH, and transfusion needs: Patients experiencing more frequent crises had lower hemoglobin, higher LDH, and consequently required a greater number of blood transfusions.

Finally, we investigated whether patients diagnosed with vaso-occlusive crises were also experiencing hemolytic crises simultaneously. Interestingly, we found a negative correlation between hemoglobin and reticulocyte count (-0.29) and LDH (-0.41). Elevated serum LDH is a marker not only for acute hemolytic crises but also for steady chronic hemolysis, showing a positive correlation with pain crisis severity and poorer outcomes. Although a study by Ballas did not find a correlation between LDH and hemoglobin, we hypothesize that elevated LDH, indicative of hyper-hemolysis, might signal lower hemoglobin levels and thus an increased frequency of pain crises (32). This finding suggests that hemolytic crises may indeed contribute to the onset of pain crises. Consequently, pain crises episodes might be better managed by monitoring early signs of hemolysis and intervening with blood transfusions, hydration, or other treatments.

5.1. Conclusions

In light of these findings, although our sample size was limited, we propose that pain crises may be mitigated by closely monitoring sickle cell patients for hemolytic markers, such as hemoglobin and LDH. These markers are not only predictive of more severe and frequent pain crises but also indicative of concurrent hemolytic events. To our knowledge, we suggest that a hemoglobin level < 7.5 and an LDH level > 1000 may serve as predictive factors for severe pain crises. Future studies with larger sample sizes are needed to confirm these results.

Footnotes

Authors' Contribution: Study concept and design: M. D.; acquisition of data: M. A. and M. R.; analysis and

interpretation of data: V. M. K. and M. A.; drafting of manuscript: M. D. and M. A.; critical revision of the manuscript for important intellectual content: V. M. K.; statistical analysis: M. A.; administrative, technical and material support: V. M. K. and M. D.; study supervision: V. M. K.

Conflict of Interests Statement: The authors declared no conflict of interests.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This study is approved by under the ethical approval code IR.SUMS.MED.REC.1399.108 .

Funding/Support: This study was not funded by any organization.

Informed Consent: All data were obtained from patient records with their consent, and no interventions were conducted in this study.

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