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Prevalence of Leg Ulcers in Sickle Cell Disease and Their Association with Disease Severity, Inflammatory, and Oxidative Stress Markers

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ABSTRACT

Leg ulcers are a devastating problem of sickle cell disease (SCD) and are associated with elevated inflammation and oxidative stress. Understanding their prevalence, severity, and biochemical associations is essential for improved management. **Objective:** To determine the prevalence and severity of leg ulcers in sickle cell disease and evaluate their association with oxidative and inflammatory stress markers, addressing the gap in the literature regarding biochemical correlates of ulcer development in SCD patients. **Methods:** This cross-sectional study was carried out at Gujranwala Teaching Hospital, Gujranwala Medical College. A total of 323 genetically confirmed SCD patients were enrolled. Clinical data, including leg ulcer characteristics and SCD severity, were documented. Serum levels of Tumor Necrosis Factor Alpha (TNF-α), Interleukin-6 (IL-6), Total Antioxidant Capacity (TAC), and Total Oxidative Stress (TOS) were quantified using ELISA. Statistical analysis was completed using SPSS-25. **Results:** The prevalence of leg ulcers was 30.0% (97/323; 95% CI: 25.1%–35.3%), increasing with SCD severity ($p<0.001$). Ulcerated patients had significantly higher TNF-α (28.5 ± 5.3 vs. 19.6 ± 4.8 pg/mL; 95% CI for difference: 7.3–10.2, $p<0.001$) and IL-6 (21.2 ± 4.7 vs. 12.9 ± 3.6 pg/mL; 95% CI: 6.9–9.6, $p<0.001$). TOS was elevated (48.7 ± 9.5 vs. 32.3 ± 7.1 µmol H_2O_2 equiv./L; 95% CI: 13.5–18.2, $p<0.001$), while TAC was lower (0.82 ± 0.14 vs. 1.27 ± 0.21 mmol Trolox equiv./L; 95% CI: −0.54 to −0.38, $p<0.001$). **Conclusions:** Leg ulcers are prevalent in SCD, particularly in patients with severe disease. SCD severity showed significant associations with inflammatory and oxidative stress markers.

INTRODUCTION

Sickle cell disease (SCD) is a collection of hemoglobinopathies categorized by existence of sickle hemoglobin (HbS). Sickle cell anemia (SCA) is the homozygous form (HbSS) of SCD [1, 2]. Hemoglobin C (HbC) inheritance alongside HbS results in hemoglobinopathy SC (HbSC) [3]. SCD manifests with systemic complications, including vaso-occlusion, stroke, pulmonary hypertension, and chronic leg ulcers (CLUs) [4]. Sickle leg ulcers (SLUs) are chronic, non-healing skin lesions. These commonly affect the lower extremities, particularly the malleolar regions [5, 6]. SLUs typically present with superficial wounds with raised borders. Sometimes these are accompanied by serous, purulent, or bloody discharge [7]. Wound characteristics include necrotic tissue due to poor vascularization, granulation tissue associated with angiogenesis, and epithelized areas indicating partial healing [8]. The size, recurrence, and duration of ulceration vary significantly, with unclear contributing factors [9].

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The pathophysiology of SLUs is multifactorial. This involves oxidative stress, vaso-occlusion, endothelial dysfunction, and chronic inflammation [10]. Neutrophils play a vital role in SCD pathology by initiating and propagating inflammatory responses and vaso-occlusive crises (VOCs) [11]. Increased neutrophil adhesion to endothelial cells leads to vascular obstruction, ischemia, and tissue damage [12]. These cells exhibit heightened activation, producing inflammatory mediators and reactive oxygen species (ROS), exacerbating oxidative stress and endothelial dysfunction [13, 14]. Excessive ROS contribute to oxidative damage in SCD, including superoxide, hydrogen peroxide, and malondialdehyde. This leads to reduced nitric oxide (NO) bioavailability, endothelial injury, and impaired wound healing [15, 16]. Imbalances between antioxidant defenses, like catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), and ROS production create a persistent oxidative environment [17]. Dysregulated cytokine production, including elevated interleukin-10 (IL-10), myeloperoxidase (MPO), and tumor necrosis factor-alpha (TNF-α), further perpetuates inflammation plus tissue damage [18]. Oxidative stress has been quantified by Total Antioxidant Capacity (TAC) and Total Oxidative Status (TOS) levels. By assessing cumulative oxidant and antioxidant activity in biological fluids [19]. The oxidative stress index (OSI) and the TOS/TAC ratio provide a comprehensive measure of oxidative burden [20]. While previous studies have examined oxidative stress markers of VOCs and hemolysis, their role in SLU pathogenesis remains underexplored [18]. Despite the significant morbidity associated with SLUs, their prevalence and severity in SCD patients remain poorly characterized. Particularly with inflammatory and oxidative stress markers.

This study aims to examine the occurrence and sternness of SLUs in patients of SCD. This also assess the impact of inflammatory and oxidative stress markers on disease progression. Current study is aimed to appraise the occurrence and severity of leg ulcers in SCD patients. The study also analyze their association with inflammatory markers (MPO, IL-10, TNF-α) and oxidative stress markers (TOS, TAC, OSI).

METHODS

A cross-sectional study was designed to measure the occurrence and leg ulcers in patients having sickle cell disease (SCD). The study investigates the impact of inflammatory and oxidative stress markers. The study was conducted at Gujranwala Teaching Hospital, Gujranwala Medical College (a constituent college of UHS, Lahore) over one year from January 8, 2024, to January 7, 2025. Ethical approval was acquired from the Institutional Review Board (IRB) of Gujranwala Medical College (Approval No IRB. 23/GMC). Informed consent, in writing, was obtained from the participants or their legal guardians before enrollment.

The sample size was designed by incorporating the Open Epi software. A confidence interval of 95% was set with 80% statistical power, based on an estimated 21% occurrence of leg ulcers in SCD patients as reported in previous studies [21]. With a 5% margin of error, the final calculated sample size was 323 patients. Patients with genetically confirmed SCD were included, and time since diagnosis was recorded for all patients to assess its association with leg ulcer severity. Only patients aged 12 years and above were included, considering the increased likelihood of ulcer development in older age groups. Patients with other causes of leg ulcers (such as venous insufficiency, diabetes, or trauma) or those receiving immunosuppressive therapy were excluded. A well-structured proforma was created to gather sociodemographic and clinical data from participants of the study and from their parents/guardians, including age, gender, time since SCD diagnosis, history of blood transfusion, hospitalization, frequency of vaso-occlusive crises, and lifetime complications. These data were verified using medical records. Socioeconomic status was classified using the Oyedeji method, which assesses parental education and occupation. The original five socioeconomic classes were merged into three categories: one is high (I and II), the second is middle (III), and the third is low (IV and V). The prevalence and severity of leg ulcers were assessed through clinical examination and patient history. The presence of active or healed ulcers was recorded, including location, number, size (in cm^2), depth, and presence of infection. Severity classification was based on ulcer size and healing status, with mild ulcers being <2 cm^2, moderate ulcers between 2–6 cm^2, and severe ulcers >6 cm^2 or with evidence of infection. The Helvaci *et al.*, method was used to evaluate SCD severity, incorporating acute chest syndrome (ACS), avascular necrosis (AVN), splenomegaly, hepatomegaly, hematocrit levels, total white cell count, and lifetime cumulative frequency of complications such as gallstones, meningitis, cerebrovascular disease, osteomyelitis, chronic leg ulcers, and priapism. Based on these scores, patients were categorized as having mild (score <8), moderate (8–17), or severe (>18) disease [22]. The number of inflammatory markers, including Tumor Necrosis Factor Alpha (TNF-α) and Interleukin-6 (IL-6), was determined employing enzyme-linked immunosorbent assay kits (Elabscience, Cat. No. E-EL-H0109 for TNF-α and Cat. No. E-EL-H6156 for IL-6). Every blood sample was taken at the initial presentation to the clinic and before starting any different treatments. The Total Antioxidant Capacity (TAC) and Total Oxidative Stress (TOS) were determined in serum samples. To determine TAC levels, orthodianisidine was bleached and detected at 660 and 870 nm with the Biolab® 310 analyzer, the results being spoken of as mmol Trolox equivalents/L. TOS levels were found from a hydrogen peroxide reference curve and reported as μmol H_2O_2 equivalents/L/L. The statistical analysis was done with SPSS version 25.0. Performances were analyzed with percentages, standard deviation and mean. An

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independent t-test was performed to look for differences in continuous variables (e.g., inflammation and oxidative stress markers) between patients with and without ulcers. Pearson's correlation coefficient was chosen to assess the way ulcer severity relates to the markers found in biological fluids. A p-value<0.05 was recognized as statistically significant.

RESULTS

The incidences of leg ulcers increased with disease severity, with 48.5% of patients with severe SCD having ulcers compared to 31.2% in moderate SCD and 15.8% in mild SCD. Out of 323 enrolled SCD patients, 97 (30.0%) had active or healed leg ulcers at the time of the study. Among these, 41 (42.3%) had mild ulcers, 36 (37.1%) had moderate ulcers, and 20 (20.6%) had severe ulcers. The incidences of leg ulcers were significantly elevated in patients with severe SCD (48.5%) in comparison to those with moderate (31.2%) or mild disease (15.8%) (p<0.001) (Table 1).

Table 1: Prevalence of Leg Ulcers among SCD Patients

SCD Severity	Total Patients (n=323)	Patients with Leg Ulcers (n=97)	Mild Ulcers	Moderate Ulcers	Severe Ulcers	p-value
Mild SCD (Score <8)	114 (35.3%)	18 (15.8%)	12 (66.7%)	5 (27.8%)	1 (5.5%)	<0.001
Moderate SCD (Score 8-17)	141 (43.7%)	44 (31.2%)	20 (45.5%)	15 (34.1%)	9 (20.4%)	
Severe SCD (Score >18)	68 (21%)	35 (48.5%)	9 (25.7%)	16 (45.7%)	10 (28.6%)	

Patients with leg ulcers were older, predominantly male, and had more frequent vaso-occlusive crises compared to those without ulcers. The patient's mean age with ulcers was 27.8 ± 6.5 years, significantly higher than non-ulcerated patients (22.3 ± 5.9 years, p=0.002). Male had an elevated prevalence of ulcers (68.0%) compared to females (32.0%, p=0.04). The frequency of vaso-occlusive crises (VOC) was significantly elevated in the ulcerated group (5.2 ± 1.4 episodes/year) compared to non-ulcerated patients (3.1 ± 1.2 episodes/year, p<0.001). Additionally, patients having leg ulcers had a high history of blood transfusions (76.3% vs. 49.6%, p<0.001) and more frequent hospitalizations in the past year (2.8 ± 1.1 vs. 1.5 ± 0.7, p<0.001) (Table 2).

Table 2: Sociodemographic and Clinical Features of SCD Patients with and without Leg Ulcers

Patient Characteristics	Ulcerated (n=97)	Non-Ulcerated (n=226)	p-value
Vaso-Occlusive Crises (Episodes/Year)	5.2 ± 1.4	3.1 ± 1.2	<0.001#
Blood Transfusion History, n (%)	74 (76.3%)	112 (49.6%)	<0.001*
Hospitalization (Last Year)	2.8 ± 1.1	1.5 ± 0.7	<0.001#

#Data are presented as mean ± standard deviation (SD) for continuous variables (independent t-test was applied); *Data are presented as number (percentage) for categorical variables (chi-square test was applied). A statistically significant p-value was considered <0.05.

Higher TNF-α and IL-6 levels indicate increased inflammation in ulcerated patients. Lower TAC and higher TOS levels suggest greater oxidative stress in patients with leg ulcers. Levels of serum of TNF-α and IL-6 were significantly elevated in ulcerated patients compared to ones who were without ulcers (p<0.001). In the same manner, TOS levels were significantly elevated in the ulcerated cluster, while TAC levels were lower (p<0.001) (Table 3).

Table 3: Inflammatory and Oxidative Stress Markers in SCD Patients with and without Leg Ulcers

Biomarkers	Ulcerated (n=97)	Non-Ulcerated (n=226)	p-value
TNF-α (pg/mL)	28.5 ± 5.3	19.6 ± 4.8	<0.001
IL-6 (pg/mL)	21.2 ± 4.7	12.9 ± 3.6	<0.001
TAC (mmol Trolox equiv./L)	0.82 ± 0.14	1.27 ± 0.21	<0.001
TOS (μmol H_2O_2 equiv./L)	48.7 ± 9.5	32.3 ± 7.1	<0.001

Data are presented as mean ± SD, and an independent t-test was applied. A p-value<0.05 was considered statistically significant.

Higher inflammatory (TNF-α, IL-6) and oxidative stress (TOS) markers correlated with increased ulcer severity, while TAC levels showed an inverse relationship. Pearson's correlation analysis depicted a strong positive correlation amongst ulcer severity and TNF-α (r=0.72), IL-6 (r=0.69), and TOS (r=0.74). Conversely, TAC levels correlated negatively with ulcer severity (r= -0.67) (Table 4).

Table 4: Correlation of Leg Ulcer Severity with Inflammatory and Oxidative Stress Markers

Parameters	r-value
TNF-α (pg/mL)	0.72
IL-6 (pg/mL)	0.69
TAC (mmol Trolox equiv./L)	-0.67
TOS (μmol H_2O_2 equiv./L)	0.74

The r-value (Pearson correlation coefficient) ranges from -1 to +1, where values nearer to +1 or -1 show stronger positive or negative correlations, respectively. A negative r-value indicates an inverse relationship.

DISCUSSION

Leg ulcers are a common yet severe complication of sickle cell disease (SCD), often connected to increased oxidative stress and inflammation. The study highlights the high occurrence of leg ulcers amongst SCD patients, particularly in those with severe disease. The overall prevalence was 30.0%, with significantly higher rates

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observed in patients with severe SCD compared to moderate and mild cases. Such conclusions are in accordance with earlier research. Ultimately, representing a direct relationship between disease severity and ulcer occurrence [21-23]. Oxidative stress has been incriminated in the pathophysiology of SCD and its complications, including leg ulcers. Our findings revealed significantly elevated total TOS levels in patients with leg ulcers. These levels are presented as compared to those without ulcers. Additionally, total antioxidant capacity TAC levels were markedly lower in patients having ulcers than non-ulcerated ones. As a result, endothelial cells do not work properly and wounds take longer to heal in people with SCD [13, 24]. Inflammation is another critical factor in the pathogenesis of leg ulcers in SCD. Patients having ulcers exhibited significantly higher points of pro-inflammatory cytokines, as well as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), compared to non-ulcerated patients. These elevated inflammatory markers may contribute to chronic vascular inflammation, delayed healing, and ulcer recurrence, as previously reported in similar cohorts [25, 26]. The correlation analysis further reinforced these associations. Ulcer severity displayed a strong positive correlation with TNF-α, IL-6, and TOS, while TAC levels correlated negatively with ulcer severity. These results propose that systemic inflammation and oxidative stress have a crucial part in ulcer progression, necessitating targeted interventions to mitigate their effects [27-29]. Apart from biochemical markers, demographic and clinical factors also influenced ulcer prevalence. Ulcerated patients were significantly older, mostly male, and experienced more frequent vaso-occlusive crises. Additionally, history of blood transfusion and hospitalizations in the past year were significantly higher among ulcerated patients, suggesting that these clinical parameters may serve as risk factors for ulcer development [30, 31]. Given the significant role of oxidative stress and inflammation in leg ulcer pathogenesis, therapeutic strategies aimed at restoring antioxidant balance and reducing inflammatory burden should be explored. Antioxidants, such as N-acetyl cysteine and omega-3 fatty acids, have shown promise in reducing oxidative stress in SCD, but further randomized controlled trials are needed to evaluate their efficacy in ulcer prevention and treatment [32, 33].

CONCLUSIONS

It was concluded that leg ulcers are a prevalent complication among patients with sickle cell disease (SCD), with their severity significantly associated with the underlying progression/ complication of SCD. Additionally, oxidative stress and inflammation play a pivotal role in the development and progression of these ulcers. Elevated levels of TNF-α, IL-6, and TOS, alongside reduced TAC levels, were significantly correlated with greater ulcer severity. These findings highlight the need for routine screening of oxidative stress markers in SCD patients with leg ulcers. Patients should be advised to follow antioxidant-rich diets and avoid factors that may trigger oxidative stress. Future research should explore interventional strategies aimed at reducing inflammation and oxidative burden to prevent or mitigate severe leg ulcers in SCD.

Authors Contribution

Conceptualization: RS
Methodology: FM, SA, FAF, RMAK
Formal analysis: MTJ
Writing review and editing: RMAK

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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