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Factors Affecting Pain Control in Patients with Sickle Cell Disease at Mwananyamala and Muhimbili Hospitals in Dar es Salaam, Tanzania

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Abstract

Background: Sickle cell disease (SCD) is the most common hemoglobin disorder in the world. Africa has the highest burden of SCD, accounting for up to 75% of the 300,000 annual births of individuals with SCD worldwide. In Tanzania, 11,000 – 14,000 babies are born with SCD each year. Despite treatment advancement, pain is still an attributable cause of admissions among patients with SCD. However, data is still lacking regarding the adequacy of pain control in patients with SCD in Tanzania. **Objective:** This study aimed to determine factors affecting pain control among patients with SCD presenting with painful events at Mwananyamala Regional Referral Hospital (MRRH) and Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania. **Methodology:** This was a cross-sectional study conducted at MRRH and MNH which are tertiary referral hospitals in Dar es Salaam, Tanzania. Patients with SCD aged 8 years and above who presented at the hospitals with painful events (from August 2022 to February 2023) were enrolled into the study. A structured questionnaire was used to collect data on participants' socio-demographic characteristics and clinical parameters. The adequacy of pain control was assessed using the WHO Pain Management Index. Multivariable binary logistic regression was used to determine factors associated with pain control. Differences were considered statistically significant when the p-value was < 0.05. **Results:** A total of 390 patients with SCD were analysed with mean age (± SD) of 15 (± 6) years. Most patients were recruited from outpatient clinics (88.2%). The male-to-female ratio was 1:1, the majority of patients had less than three pain episodes per year (77.9%), and most patients presented to the hospital with mild pain (64.6%) and were on Hydroxyurea (62.3%). Furthermore, one-third of patients had inadequate pain control. Factors associated with inadequate pain control included receiving initial pain management in other health facilities (adjusted odds ratio [aOR] and 95% confidence interval [CI] = 2.5 (1.5- 4.5), p=0.001), presenting to the hospital with moderate pain (aOR = 2.2, 95% CI [1.3-3.8], p=0.006), and

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presenting to the hospital with a fever (aOR = 3.8, 95% CI [1.1 – 13.9], p=0.04). Having severe pain and receiving initial treatment at MRRH or MNH seemed to be protective factors (aOR = 0.33, 95% CI [0.11- 0.97], p=0.04, and aOR = 0.29, 95% CI [0.14 -0.61], p=0.001, respectively).

Conclusion: A considerable proportion of patients with SCD receive sub-optimal pain control. Receiving initial pain management from other healthcare facilities, presenting to the hospital with moderate pain, and having a fever were associated with inadequate pain control. Further research is warranted to elucidate ways of optimising the management of pain in patients with SCD in Tanzania.

Keywords: Sickle cell disease; pain severity; pain control; hospital; Tanzania

Introduction

Sickle cell disease (SCD) is a genetic disorder of the red blood cells (RBCs) resulting from a point mutation that leads to substitution of valine for glutamic acid at position 6 of the β-globin chain [1,2]. Vaso-occlusive (VOC) phenomena and hemolysis are the clinical hallmarks of SCD. VOC results in recurrent painful episodes and a variety of organ system complications that can lead to lifelong disabilities [3–5]. In Tanzania, 11,000 – 14,000 babies are born with SCD each year [6,7].

Pain is the defining feature of SCD and the leading cause of hospitalisation [8,9]. Pain in patients with SCD typically occurs from the first year of life, concomitant with the fall in levels of fetal haemoglobin. Unlike sickle haemoglobin (HbS), fetal haemoglobin (HbF) can resist sickling when subjected to changes such as hypoxia, which usually triggers pain; hence, its presence in high levels protects against sickling [6]. Pain in patients with SCD varies from acute, chronic to neuropathic pain [10,11].

Despite advances in treatment and the introduction of disease-modifying drugs such as Hydroxyurea, pain remains the most common cause for hospitalization among SCD patients [1,7,12]. Studies have indicated that patients with SCD frequently suffer from both under-treatment and overtreatment of their pain in hospitals [13–15]. Adequate pain control involves several measures, including adequate hydration, choosing suitable pain medication based on the World Health Organization (WHO) analgesic ladder and treatment of any underlying cause for the pain if present [16]. Analgesia should be administered to SCD patients within 30 minutes of hospital admission, and patients should be reviewed after receiving analgesia to assess its effectiveness [4,6,17,18]. Adequate pain control is attainable if suitable analgesia is used at the proper dose and frequency and is administered per the WHO analgesic ladder alongside proper management of hydration [18]. According to the WHO, there are three levels of pain: mild pain, moderate pain and severe pain [10,19] Appropriate analgesia for mild (level one) pain is non-opioid medication such as paracetamol or non-steroidal anti-inflammatory drugs (NSAIDs) plus adjuvant therapy such as massage and pain distraction therapy such as television. Level two (mild to moderate) pain is managed by weak opioids such as codeine plus or minus non-opioid medication, and level three (severe pain) is managed by strong opioids such as morphine [4,7].

The adequacy of pain management among patients with SCD in Tanzania is yet to be fully explored. This study aimed at exploring patients' experiences and factors associated with adequate pain control among patients with SCD in Dar-e-salaam, Tanzania, with the goal to inform measures for optimization of the management of pain in this patient population.

Methodology

Study Design and Study Setting

This was a hospital-based cross-sectional study conducted at Mwananyamala Regional Referral Hospital (MRRH) and Muhimbili National Hospital (MNH) in Dar-es-salaam, Tanzania.

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MNH is the only national Hospital in Tanzania, located in the Ilala district and offers SCD-integrated care to adult and pediatric patients, attending about 100-150 patients per week. The clinics are on Thursdays for paediatrics and Fridays for adults at the general haematology clinics. The hospital also offers inpatient care, and about 10-20 patients with SCD are admitted monthly; nevertheless, emergency services are available for those presenting with acute SCD pain. The hospital has adequate physicians, haematologists, paediatricians, pharmacists, and nurses who provide specialized care to all patients with SCD. MRRH is a tertiary-level hospital located in Kinondoni district and provides integrated SCD care, with regular SCD outpatient clinics on Tuesdays, attending about 30-40 patients with SCD per week. The hospital is capable of providing emergency services and inpatient care.

Besides attending to SCD patients during regular clinic days, both hospitals also attend to SCD patients referred from other hospitals as well as self-referral patients who come directly from home. Hydroxyurea is readily available for patients attending these two hospitals, and all-important paid medication including opioid analgesics, NSAIDs and paracetamol are available. Both the MRRH and MNH are part of the Sickle Pan-African Research Consortium (SPARCO)-Tanzania project that maintains electronic registry for patients with SCD who are regularly seen at these and 12 other health facilities across 4 administrative regions in Tanzania.

Sample Size and Sampling Method

Study participants were all patients with SCD, aged 8 years and above, who presented at MRRH and MNH with painful events from August 2022 to February 2023.

The sample size required for this study was calculated by using the formula described by Kish Lesley.

$$n = \frac{z^2 p(1-p)}{e2}$$

Z = level of confidence (1.96 for 95% confidence level), ε = margin of error, which is 5% and p = proportion with adequate pain control, which was taken as 50% due to the lack of similar studies that assess the adequacy of pain control in patients with SCD.

$$n = \frac{1.96^2 * 50(100-50)}{52}$$

n=384

Participants in the study were recruited consecutively via purposive sampling from clinics and inpatient wards until the desired sample size of 384 individuals was attained.

Data Collection Methods

Data was collected at MRRH and MNH by the principal investigator (PI) and a trained research assistant. For patients attending SCD clinics, the PI explained the study and inquired whether they were experiencing sickle cell-associated pain that day or not. Only those who had pain and consented were enrolled into the study. For inpatients, the attending nurses and clinicians were informed about the study and notified the PI via phone whenever patients with SCD pain were admitted. The PI then attended to the patients and informed patients about the study and its advantages, obtained written consent/assent and enrolled patients who agreed to participate in the study. Critically ill patients, such as those admitted in the intensive care unit (ICU), patients with documented neurological conditions such as dementia, cognitive impairment, and psychosis, and those with identifiable other causes of pain were excluded from this study. A physical examination and hydration status assessment were also performed on each patient, and vital signs were taken, including pulse rate, blood pressure and temperature. Other clinical characteristics were obtained from the patients' medical records (SCD Health Passport).

A structured questionnaire was used to collect data. The questionnaire had three parts: The first part collected data on social-demographic characteristics of the patient, including the patient's number (code), date of birth, gender, weight, residence, marital status, education level, number and

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frequency of admissions due to painful events, steady-state hemoglobin, weight, and medication history. The second part incorporated the standardized age-appropriate pain assessment tool, where level 0 was interpreted as no pain, level 1- 3 was mild pain, level 4-7 was moderate pain and level 8-10 was interpreted as severe pain. For the pediatrics and young adults, we used the Numeric Pain Rating Scale, while in adults aged 18 years and above, the Brief Pain Inventory Scoring Short Form was used [20,21]. The third part of the questionnaire recorded assessment of the hydration status and adequacy of pain control which was assessed within 12-72 hours from the first dose of analgesia using the Pain Management Index [22] where any negative number on the pain management index indicated inadequate pain control, 0 and numbers above it (positive numbers) was interpreted as the appropriate management for the level of pain [20,22].

Statistical Analysis

The data collected were sorted and checked for completeness and consistency. Data were de-identified, coded, cleaned, and entered into Statistical Package for Social Sciences (SPSS) version 23 for analysis. The clinical characteristics and laboratory parameters of patients presenting with pain were analyzed using a frequency distribution table and percentages. The proportion of patients with SCD who had adequate pain control was obtained. The association between the adequacy of pain management and the independent variables was determined by cross-tabulation using the Pearson Chi-square or Fisher's exact test as appropriate. All factors with a p-value <0.2 in the bivariate analysis were entered into a multivariable binary logistic regression analysis to determine the independent factors associated with inadequate pain control. Results were considered statistically significant when the p-value was less than 0.05.

Ethical Consideration

The study obtained ethical approval from Muhimbili University of Health and Allied Sciences (MUHAS) Institutional Review Board (IRB), number "MUHAS-REC-10-2022-1420" and separate permissions from MRRH and MNH. Written informed consent/assent was obtained for each participant. No participant names or identities were recorded; each study participant was coded using numbers. De-identification at data entry was done to minimize the direct association of patients with their data. Only personnel directly involved with the research had access to the data. Patients' confidentiality was further enhanced by storing all the collected information in a locked cabinet and in a computer with a secure password.

Results

Socio-Demographic and Clinical Characteristics of the Study Participants

A total of 400 patients with SCD met the inclusion criteria. Of these, 10 were excluded as follows: two were admitted to the ICU, where one had convulsions that led to him being sedated, and another had pain due to skin reaction secondary to antibiotic use (Amoxicillin), and eight patients did not consent to participate in the study (Figure 1).

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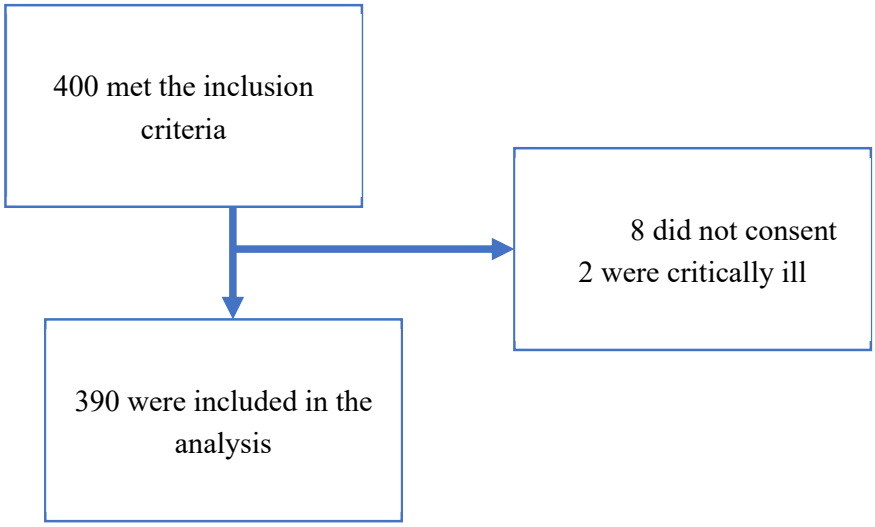

Figure 1. Flow chart diagram of patients enrolled in the study.

Most of the study participants 344/390 (88.2%) were enrolled from outpatient settings. The mean age of the participants was 15 years, and there was a balance between males and females (1:1 ratio). Further, 166/390 (42.6%) participants were residing outside of Dar es Salaam. Over two-thirds of participants, 304/390 (77.9%), experienced pain under three episodes annually, whereas 86/390 (22.1%) had three or more episodes. The majority of participants, 252/390 (64.6%), reported mild pain, and the remaining one-third suffered moderate to severe pain. Two-thirds of patients were on Hydroxyurea, and 16 patients presented with fever. Out of the 16 patients who presented with fever, 10 were under 5 years of age (Table 1).

Table 1. Baseline socio-demographic and clinical characteristics of the study participants.

Variable	Mean ± SD	Frequency (%), N= 390
Age(years)	15 ± 6	
≤ 14		230 (59.0)
>14		160(41.0)
Residence		
Dar es salaam		224 (57.4)
Outside Dar es salaam		166 (42.6)
Hb- Steady state(g/dl)	7.6 ± 1	
Hb on recruitment (g/dl)		
Below steady state		35 (9.0)
Steady state		355 (91.0)

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• **Number of pain episodes per year**	•
• <3	• 304 (77.9)
• ≥ 3	• 86 (22.1)
• **Pain scale**	•
• Mild pain (Level 1-3)	• 252 (64.6)
• Moderate pain (Level 4-7)	• 103 (26.4)
• severe pain (Level 8-10)	• 35 (9.0)
• **Hydroxyurea use**	•
• Yes	• 243 (62.3)
• No	• 147 (37.7)
• **Body temperature (^{0}c)**	•
• ≤37.6^{o}c	• 374 (95.8)
• >37.5^{o}c	• 16 (4.2)

Factors Associated with Inadequate Pain Control

Among the SCD patients enrolled in our study, we found that 31% (121/390) had inadequate pain control. Upon bivariate analysis, there was more inadequate pain control among those aged above 14 years compared to those 14 years and below (36.9% vs. 27.0%, p = 0.04). Patients who lived outside Dar es Salaam were more likely to have inadequate pain control compared to those who lived in Dar es Salaam (40.4% vs. 24.1%, p=0.001). Having moderate pain was associated with inadequate pain control compared to those with mild or severe pain (52.4% vs. 24.6% vs. 14.3%, p<0.001). Patients who were initially treated at home or in other healthcare facilities were more likely to have inadequate pain control compared to those initially treated at MRRH/MNH (31.8% vs. 49% vs. 9.9%, p<0.001). Furthermore, although not statistically significant, patients who were not on Hydroxyurea and those who presented to the hospital with fever were more likely to have inadequate pain control compared to their counterparts (36.7% vs. 27.6%, p = 0.07 and 50.0% vs. 30.2%, p = 0.09, respectively) (Table 2).

Table 2. Factors associated with inadequate pain control among patients with SCD who presented with pain.

Variable	Adequacy of Pain Control		
	Adequate	**Inadequate**	**p-value**
Age (Years)			
≤ 14	168 (73.0)	62 (27.0)	
>14	101 (63.1)	59 (36.9)	**0.04**
Gender			

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Male	134 (69.8)	58 (30.2)	
Female	135 (68.6)	63 (31.8)	0.74
Education			
Primary or below	160 (71.1)	65 (28.9)	
Secondary or above	109 (66.1)	56 (33.9)	0.32
Residence			
Dar es Salaam	170 (75.9)	54 (24.1)	
Outside Dar es Salaam	99 (59.6)	67 (40.4)	**0.001**
Recruitment Hb (g/dl)			
Below steady state	22 (62.9)	13 (37.1)	
Steady-state	247 (69.6)	108 (30.4)	0.45
Pain episodes per year			
<3	209 (68.8)	95 (31.3)	
≥ 3	60 (69.8)	26 (30.2)	0.89
Pain scale			
Mild pain (Level 1-3)	190 (75.4)	62 (24.6)	
Moderate pain (Level 4-5)	49 (47.6)	54 (52.4)	
Severe pain (Level 6-10)	30 (85.7)	5 (14.3)	**<0.001**
Site of maximum pain			
Extremities	17 (77.3)	5 (22.7)	
Head and neck	89 (63.1)	52 (36.9)	
Multiple sites	110 (72.8)	41 (27.2)	
Trunk	53 (69.7)	23 (30.3)	0.3
Hydroxyurea use			
Yes	176 (72.4)	67 (27.6)	
No	93 (63.3)	54 (36.7)	0.07
Fever (≥37.5ºc)			
No	261 (69.8)	113 (30.2)	
Yes	8 (50.0)	8 (50.0)	0.09
Hydration status			
Well hydrated	254 (69.8)	110 (30.2)	
Dehydrated	15 (57.7)	11 (42.3)	0.19
Site of initial pain management			
Home	75 (68.2)	35 (31.8)	
Other healthcare facilities	76 (51.0)	73 (49.0)	**<0.001**
MNH/MRRH	118 (90.1)	13 (9.9)	
Steady HB(g/dl)			
≤7	163 (68.8)	74 (31.2)	
>7	106 (69.3)	47 (30.7)	1.00

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Independent Factors Associated with Inadequate Pain Control

To ascertain independent predictors of inadequate pain control, we performed multivariable binary logistic regression analysis. Patients who lived outside Dar es Salaam were 74% more likely to have inadequate pain control compared to those who lived in Dar es Salaam (aOR = 1.74, 95% CI [1.1-2.9], p = 0.03). Presenting to the hospital with moderate pain increased the odds of inadequate pain control 2.2-fold as compared to those presenting with mild pain (aOR = 2.2, 95% CI [1.3-3.8], p=0.006). On the contrary, presenting to the hospital with severe pain reduced the odds of having inadequate pain control by 67% compared to presenting with mild pain (aOR = 0.33, 95% CI [0.1- 1.0, p = 0.04). Patients presenting to the hospital with a fever were 3.8 times more likely to have inadequate pain control compared to those presenting without a fever (aOR = 3.8, 95% CI [1.1-13.9], p=0.04). Compared to receiving initial treatment at home, receiving initial pain management at other healthcare facilities increased the odds of inadequate pain control 2.5 folds (aOR 2.5, 95% CI [1.5-4.5], p = 0.001), while receiving initial treatment at MRRH/MNH reduced the odds of having inadequate pain control by 71% (aOR 0.29, 95% CI [0.1 -0.6], p<0.001) (Table 3).

Table 3. Multivariable logistic regression for the factors associated with inadequate pain control.

Variables	Crude OR	p-value	Adjusted OR (95% CI)	p-value
Age (years)				
≤ 14	Ref		Ref	
>14	1.6 (1 -2.4)	0.04	1.3 (0.8 – 2.1)	0.36
Residence				
Dar es salaam	Ref		Ref	
Outside Dar es salaam	2.1 (1.4 – 3.3)	0.01	1.74 (1.1-2.9)	**0.03**
Pain scale				
Mild pain	Ref		Ref	
Moderate pain	3.4 (2- 5.5)	<0.001	2.2 (1.3- 3.8)	**0.006**
Severe pain	0.5 (0.2- 1.4)	0.18	0.33 (0.1- 1.0)	**0.04**
Hydroxyurea use				
Yes	Ref		Ref	
No	1.5 (1.0 – 2.4)	0.07	1.42 (0.9- 2.4)	0.18
Fever				
No	Ref		Ref	
Yes	2.3 (0.8 -6.5)	0.1	3.8 (1.1 – 13.9)	**0.04**
Site of initial pain management				
Home	Ref	0.006	Ref	
Other healthcare facility	2 (1.2- 3.4)	<0.01	2.5 (1.5- 4.5)	**0.001**
MRRH/MNH	0.3 (0.1- 0.5)		0.29 (0.1 -0.6)	**<0.001**

Discussion

To the best of my knowledge, this is the first study with a large sample size conducted on factors affecting the adequacy of pain control among patients with SCD in Tanzania. We show that one-third of patients with SCD presenting at tertiary-level hospitals with pain had inadequate pain control. Residing outside Dar es Salaam and receiving initial treatment at hospitals other than the MRRH and MNH study sites, as well as presenting to the hospital with moderate pain or fever, were associated

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with inadequate pain control, while presenting with severe pain appeared to be a protective factor. This study provides critical insights into factors associated with the adequacy of pain control in one of the settings with the highest burden of SCD in Sub-Saharan Africa and could inform measures to optimize pain management in this patient population.

Living outside Dar es Salaam was associated with an increased chance of inadequate pain control. The possible explanation for this observation is that there is more specialised care in Dar-es-Salaam than in other regions. However, further studies are needed to assess what factors contribute to this discrepancy. Studies have shown that adult patients with SCD are likely to visit multiple hospitals for treatment of their acute pain[23] This could be the reason for to seek medical care at MRRH and MNH, despite receiving care in other hospitals.

We observed that patients who presented with moderate pain had two-fold higher odds of having inadequate pain management. This could be because most patients were enrolled in the clinic and ended up receiving pain medication in outpatient setting where parenteral medication for pain and IV fluids are not normally administered. The initiation of outpatient infusion centres (IC) where patients could receive IV fluids and parenteral pain medications, as is being conducted in other countries such as Uganda [6], will enable patients with moderate pain to receive parenteral medication which will improve the adequacy of pain management in outpatient settings. The American Society of Haematology recommends that patients with SCD who present to the hospital with pain should be given analgesia within one hour of arrival at the hospital. It also recommends pain reassessment to be done within 30 minutes after the initial dose of analgesia[24].

This study showed almost a four-fold increase in odds of inadequate pain control for patients who presented to the hospital with a fever. This association highlights infection as the major trigger for pain in patients with SCD and calls attention to proper management of infection for adequate pain relief to be achieved. A study done by Makani et al at MNH found that fever was the second most common cause of admission, especially in children under 5 years of age who had SCD [7]. This was similar to the observation from our study were nearly two thirds of the patients who had fever were under 5 years of age. Receiving pain control at MRRH and MNH seemed protective in terms of adequacy of pain control as opposed to treatment received in other healthcare facilities. This could be due to the availability of specialised care and access to relatively more pain medications, including Morphine, at MRRH and MNH.

The majority of patients with SCD who presented with painful events at MRRH and MNH attained adequate pain control. However, this study found that about one-third of patients had inadequate pain control. This was slightly lower compared to another study done in Uganda, which showed that a larger percentage of children [65.6%) who presented with SCD-related painful events had inadequate pain control[6]. The study in Uganda included only children attending outpatient SCD clinics, contrary to our study, which included both children and adults in both outpatient and inpatient settings. Further, two-thirds of patients reported having only mild pain. This could be because, by the time they arrived at MRRH and MNH, they had previously received pain medication either at home or at another healthcare facility, and only one-third of patients received their initial pain management at MRRH and MNH. A study done in Tanzania by Mkoka et al showed that most patients with SCD, in coping with day-to-day painful events that are related to SCD, use “self-care remedies” for pain control [25]. This was similar to our study, where a significant number of patients self-medicated for pain at home. Patients who received pain medication at home were adequately managed compared to those who received pain medication at facilities other than MRRH and MNH. We speculate that this because the pain treated at home is mostly mild pain, which can be controlled by over-the-counter pain medications such as Paracetamol. Our study highlights the need to educate patients with SCD on home-based pain interventions for SCD pain[26].

Although individuals who were on Hydroxyurea appeared more likely to have adequate pain control, this observation did not reach statistical significance in our study, despite the known effects of Hydroxyurea in disease modification[15,27]. A study by Guillaume showed that the amount of fetal haemoglobin has a direct effect in reducing the morbidity and mortality associated with SCD

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and episodes of painful crisis. However, its role is limited once a painful event has occurred[28,29]. In our study, two-thirds of patients presented with pain affecting multiple sites, in contrast to observations in the study done in Uganda, where most patients had pain in their extremities[6]. Another study by Linda Frank indicated that children with diffuse pain had longer periods of hospital stay with no association with pain scales[18,30]. The most prevalent region of pain in Linda's study was the lower back, followed by the abdomen, legs, and chest, while in this study, the majority of patients had pain in multiple sites [18]. A study by McClish found that pain located on the chest was most commonly associated with hospital admissions than pain in other sites. This was due to the high likelihood of being acute chest syndrome[31].

The majority of those who had inadequate pain were aged above 14 years. This was comparable to a study done in the UK, which observed that the VOC rate and other complications tend to gradually rise with advancing age in SCD patients. Another study found that older children had longer painful episodes, with no changes in frequency or intensity[32–34]. Our study did not find association between age and an increase in the frequency of pain crises. Despite the absence of direct association in this study, other studies have shown that pain episodes increase with age and are more perceived in adolescents than in young children. In the majority of pediatric patients, the pain is reported by caretakers rather than patients themselves [12].

Our study had several limitations. First, recall bias may have affected the reported pain episodes as most of the pain information was patient-reported. Secondly, we were unable to establish the causes of inadequate pain control, as the study focused on ascertaining the factors affecting pain control. Also, the study was limited by the inability to assess changes in variables over time. Future studies should evaluate the adequacy of pain control in primary and tertiary-level healthcare facilities in prospective cohorts of SCD patients.

Conclusions

A third of patients with SCD at tertiary healthcare facilities in Dar-es-salaam reported sub-optimal pain control. The significantly associated factors were residing outside Dar-es-salaam, receiving initial pain management from other healthcare facilities, presenting to the hospital with moderate to severe pain, and having a fever. Further research is warranted to elucidate ways of optimising the management of pain in patients with SCD in Tanzania.

Author Contributions: Happiness Joseph, Lulu Chirande, Mbonea Yonazi and Ritah Mutagonda designed the study, collected data, analysed the data and drafted the first manuscript. Avelina Mgasa, Mwashungi Ally, Clara Chamba, Ahlam Nasser, William Mawalla, Magdalena Lyimo, Benson R. Kidenya, Agnes Jonathan, Florence Urio, Paschal Ruggajo and Emmanuel Balandya participated in data analysis and reviewed the manuscript. All authors approved the final version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest. Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data processed in this study are available on request from the corresponding Author due to privacy". Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the MUHAS

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Research and Ethics Committee (protocol code MUHAS - REC -10-2022-1420 and the reference letter of DA 282/298/01.C/1420, and date of approval 27/10/2022).”

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