



Original Article

Adult Sickle Cell Anaemia Patients in Bone Pain Crisis have Elevated Pro-Inflammatory Cytokines

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Abstract. Background and Objectives: Inflammatory markers that influence bone pain crisis (BPC) and other complications of sickle cell anaemia (SCA) are numerous and play various roles. This study determined the plasma levels of tumour necrosis factor (TNF) - α , interleukin - 8 (IL-8), and endothelin - 1 (ET-1) in adult SCA patients during BPC and in steady state. In addition, the plasma levels of these cytokines were correlated with the severity of BPC of the patients.

Methods and Materials: Sixty adult SCA patients (30 during BPC and 30 during steady state) and 30 haemoglobin A controls were enrolled for this cross-sectional study. The severity of BPC was assessed clinically, and questionnaires were filled. Plasma levels of TNF- α , IL-8 and ET-1 were quantified by ELISA, and haematological parameters were determined using a 5-part auto-analyzer. Plasma levels were correlated with the severity of bone pain crisis. Results were considered statistically significant if $p < 0.05$.

Results: Plasma TNF- α , IL-8, and ET-1 were significantly elevated in the BPC group than in the steady state group and the controls. Plasma TNF- α , IL-8 and ET-1 were markedly higher in the severe BPC groups than the steady state and control groups, There was a positive correlation between TNF- α and ET-1 in the bone pain crisis group.

Conclusion: Elevated levels of plasma TNF- α , IL-8, and ET-1 further establish the chronic inflammatory state in SCA and equally affirm their significant contribution, not only to pathogenesis but also to the severity of pain in SCA.

Keywords: Sickle cell anaemia, Cytokines, Bone pain crisis, Severity, Steady state.

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Introduction. Vaso-occlusive crisis (VOC) is the commonest acute presentation of sickle cell anaemia (SCA) patients.¹ Bone pain crisis (BPC) in SCA is the most prevalent form of VOC, and it is the specific term for VOC affecting bones; thus BPC is used in this literature.² BPC is an acute episodic painful crisis that results from microcirculatory obstruction by sickle erythrocytes

leading to ischaemic-reperfusion injury of bone and necrosis of bone marrow. Bone marrow necrosis is accompanied by the release of several inflammatory mediators.² In addition to other functions, inflammatory mediators have the ability to bind specific nociceptive receptors on neurons of peripheral nerves. Hence, inhibition of secretion or neutralization of these peptides would

ameliorate pain.³ Episodes of BPC in SCA patients are highly variable, ranging from one to six per year and up to 10 events per year for some homozygous patients depending on certain environmental and genetic factors.¹ Following haemolysis, released haem contributes to activation of leucocytes, platelets and endothelial cells. This activation induces nuclear factor kappa B (NF- κ B), signal transduction and transcription 3 (STAT3), and other transcription factor pathways to increase production of pro-inflammatory and anti-inflammatory cytokines. Up-regulation of these transcription pathways leads to an imbalance between the pro- and anti-inflammatory cytokines, that is characteristic of the chronic inflammatory state seen in SCA patients.⁴⁻⁶ Recently, studies have shown that targeting a specific inflammatory pathway may be sufficient to reduce vaso-occlusion and serve as potential therapeutic options.^{7,8}

Pro-inflammatory mediators have been extensively studied in SCA, but there is a paucity of information that relates the cytokines to the severity of bone pain crisis. TNF- α is a pro-inflammatory cytokine that stimulates tumour necrosis via its receptors, TNFR55 and TNFR75.⁹ TNF- α is produced mainly by monocytes/macrophages and to a less extent by T-cells, smooth muscle cells, adipocytes, and fibroblasts.¹⁰ TNF- α worsens vaso-occlusion in SCA by enhancing endothelial adhesiveness, activating leucocytes, and coagulation cascade.¹⁰⁻¹² Several studies have shown altered levels of some cytokines in SCA patients.¹²⁻²⁰ Some found a significantly higher level of TNF- α in SCA patients in VOC and/or during steady state than in healthy HbA controls.¹⁶⁻¹⁸ Contrarily, Tavakkoli et al. and Graido-Gonzalez et al. demonstrated insignificantly elevated TNF- α level in SCA patients in VOC compared with patients in the steady-state group and controls.^{13,21}

Interleukin-8 (IL-8) is a CXC chemokine that stimulates endothelial cell proliferation and angiogenesis through its receptors (CXCR1 and CXCR2), which are expressed mainly by neutrophils.^{20,22} IL-8 is produced by neutrophils, endothelial cells, macrophages, fibroblasts, and platelets. IL-8 activates re-arrangement of the cytoskeleton, changes in intracellular Ca⁺⁺ levels, integrins, promotion of protein-granule exocytosis, and respiratory burst in neutrophils.^{23,24} IL-8 contributes to the initiation and propagation of

inflammation by activating neutrophils, which are the first line recruits to the site of vascular injury. Similar to the role of IL-1 and TNF- α , IL-8 increases the endothelial adhesiveness of the sickle red cells leading to impairment of microcirculation and exacerbating painful episodes.^{11,15,25} The most potent vasoconstrictor known, endothelins (ET), are a family of 21 amino acid peptides and ET-1 is the most prevalent subtype of the four subtypes characterised.²⁶ ET-1 constricts large vessels, resistance arterioles and even post-capillary venules, the usual site of vaso-occlusion in SCA.²⁷ Endothelin is produced by endothelial cells at a steady rate that is increased following endothelial injury or activation. The release of ET-1 is modulated by TNF- α and other inflammatory mediators.²⁸ Therefore, the ET-1 level is expectedly elevated in SCA patients because of the ongoing endothelial activation and elevated pro-inflammatory cytokines. Endothelins act via two specific G-protein-coupled membrane receptors, ETR-A and ETR-B, which are on vascular smooth muscle cells and the smooth muscle contraction results from inositol-triphosphate-mediated increases in intracellular calcium. Though ET-1 is rapidly internalised and cleared from circulation by the lungs within minutes, its vasoconstrictive effect lasts as long as 1 hour.²⁹ In *in vitro* assays, endothelin stimulated monocyte production of inflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-8 etc.), neutrophil production of platelet-activating factor (PAF). ET-1 also enhances monocyte and neutrophil chemotaxis.³⁰⁻³² Endothelins upregulate endothelial cell expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, which are adhesion molecules that participate in the recruitment of leukocytes to sites of inflammation.³³ Conversely, neutrophil proteases play a crucial role in cleaving bioactive ET-1 from its precursor molecule, thereby leading to the production of active ET and resulting in a vicious cycle with worsening inflammation.^{21,34} We, therefore, hypothesize that pro-inflammatory mediators would increase with the severity of bone pain in SCA patients. The aim of our study was to determine the plasma levels of TNF- α , IL-8 and ET-1 in SCA patients during bone pain crisis compare with those of SCA patients in steady state and to correlate these with the severity of pain.

Materials and Methods.

Study participants. This cross-sectional study consisted of 90 adult individuals enrolled and divided into three groups as follows - Bone pain crisis (BPC) group made up of 30 SCA patients enrolled consecutively at presentation during acute bone pain crisis at the Haematology day care unit (HDCU) of University College Hospital (UCH), Ibadan, South-West Nigeria. BPC was defined as the occurrence of pain in the extremities, back, and/or chest (ribs and/or sternum) that led to a hospital presentation, and could not be explained except by sickle cell anaemia;^{1,2} Steady state (steady) group made up of 30 SCA patients enrolled during routine follow up visit. Steady state was defined as stable health state in SCA patients who did not have bone pain or any other crisis and no blood transfusions in the previous two months,¹⁸ and Control (HbA) group composed of 30 HbA individuals who were students and workers in the study hospital. The control participants were healthy (HbA) age- and sex-matched adults without previous clinical evidence of haemoglobinopathies. The patients (in both BPC and steady groups) were diagnosed according to their haemoglobin profile as having homozygous haemoglobin S (HbSS) by alkaline electrophoresis and High-Performance Liquid Chromatography (HPLC). The control participants were confirmed as having haemoglobin A (HbAA) by HPLC. The individuals with concurrent overt infection, other acute complications than bone pain crisis, pregnancy, other haemoglobinopathies, and those on hydroxyurea (HU) were excluded.

The researcher/attending Physician interviewed all SCA participants, and questionnaires were completed. The survey contained sections on bio-data, past medical history obtained from the patients' notes, and clinical assessment for the management of the bone pain crisis. University of Ibadan/University College Hospital ethics review committee approved the study (UI/EC/14/0089), and all participants gave written informed consent.

Clinical severity assessment. With the aid of a questionnaire, all SCA patients were assessed for bone pain after clerking for general and organ-specific signs and symptoms. Pain assessment included pain site (ribs, sternum, back, lower or upper limbs), pain duration (up to 4 days; 5-7 days or more than 7 days), and pain intensity based on single dimensional verbal pain numerical rating

system (that is the patient's perception of pain on a scale of 1-10). The grading of pain was essential because its grade guided the type or class of analgesia administered to abate the pain. The pain was managed as mild for a verbal, numerical score 1-4; as moderate for a score of 5-6; and as severe for a score of 7-10.^{35,36} However, for this study, the assessment of patient's pain included the doctor's perception of the patient's pain. This aspect had questions on behaviour of patient during painful episode (normal, agitated, very disturbed, or too quiet) and the analgesic used to abort the pain (Paracetamol; Non-Steroidal Anti-inflammatory Drugs [NSAIDs]; Opioids; patient-controlled analgesia [PCA]; or intensive care unit [ICU] care) adapted from WHO analgesic ladder.^{25,37}

For ease of comparability of variables in this study, patient's and doctor's perceptions were analyzed and summarized as total summary pain score (TSPS). TSPS was adapted for this study and calculated as follows:

$$TSPS = [patient's\ pain\ score\ \times\ duration\ of\ pain] + [patient's\ behaviour] + [analgesia\ used]$$

Each characteristic of patient's pain was scored as follows: pain intensity was accorded 1, 2 or 3 respectively for verbal, numerical score 1-4, 5-6; 7-10 respectively. Duration of pain prior to patient's presentation accorded 1, 2 or 3 for a pain that was respectively less than 4 days, or lasted 5-6 days; or lasted more than 7 days. The same was done for the immediate analgesic intervention to abate the pain: accorded 1, 2, or 3 respectively for Paracetamol/NSAIDs; Opioids; or for the pain that necessitated patient-controlled analgesia/intensive care. Patient's behaviour attracted a maximum of four (4) for a patient who was too quiet, 3 for a very disturbed patient, 2 for an agitated patient and 1 for a patient who appeared normal.

Venous blood was collected from all the participants at the time of presentation to the hospital and dispensed into two EDTA vacutainers. One for analysis of the haematological parameters, the second tube was centrifuged, and plasma was stored in aliquots at -20°C until cytokines were assayed.

Haematological parameters. Complete blood count (CBC) was performed using Sysmex XS - 1000i (Sysmex Corporation, Kobe, Japan), a fully automated 5-part counter.

Plasma Cytokine Assays. Plasma TNF- α , IL-8 and ET-1 were quantified using high-sensitivity commercial enzyme-linked immunosorbent assay (ELISA) kits (Span[®] Biotec Limited, Shenzhen, China) in accordance with the manufacturer's instructions.

Statistical analysis. Data were analyzed using SPSS version 22.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill.). The descriptive data were presented as means \pm standard deviation except otherwise stated. Frequencies were shown in tables and graphs. Kruskal-Wallis test was used to compare means of the independent variables. Significant results were subjected to post hoc analyses for pairwise comparisons. Spearman rho analysis was performed for correlation of the haematological parameters and/or cytokines with the severity of bone pain crisis. Results were considered statistically significant if $p < 0.05$.

Results.

Demographic and haematological parameters of the participants. Demographic characteristics of the study participants, as well as the haematological parameters, are summarized in **Table 1**. Of the 90 adults evaluated, there were 30 SCA patients (15 males and 15 females) in bone pain crisis, 30 (12 males and 18 females) SCA patients in steady state and 30 HbA controls (12 males and 18 females). While the total leucocyte and platelet counts were significantly elevated in the BPC compared to those of HbA controls

($p=0.000$ in each case), the haematocrit was significantly lower in both SCA groups than the HbA ($p=0.000$).

Plasma levels of TNF- α , IL-8 and ET-1 in sickle cell anaemia patients and haemoglobin A controls. The plasma levels of TNF- α , IL-8 and ET-1 of the different groups were compared in **table 2**. The mean plasma TNF- α was significantly higher in the BPC group (373.78 ± 354.95 ng/L) than in the steady state group (99.77 ± 92.86 ng/L, $p=0.000$), and in the HbA controls (82.20 ± 29.32 ng/L, $p=0.000$). Similarly, the mean plasma IL-8 level was significantly higher in the BPC group (464.11 ± 475.99 ng/L, $p=0.005$) than in the steady state group (233.57 ± 294.35 ng/L, $p=0.005$) and in the HbA group (183.92 ± 198.58 ng/L, $p=0.002$). The mean plasma ET-1 was significantly higher in the BPC group (164.90 ± 214.76 ng/L) than in the steady state (56.16 ± 48.34 ng/L, $p=0.038$) and in the HbA groups (51.18 ± 28.70 ng/L, $p=0.042$).

Severity of bone pain crisis in the bone pain crisis group. **Table 3** summarizes the clinical severity of bone pain crisis in the SCA patients at presentation. Of the 30 patients in the BPC group, a majority 15 (50%) reported a verbal, numerical pain score of 7-10 while two (6.7%) reported a mild pain score of 1-4 and the remaining 13 reported a moderate score of 5-6. Half (15) of the BPC patients reported that they had experienced bone pain for a few hours to 4 days and nine (30%) had experienced pain for 5 to 7 days. Six

Table 1. Demographic and haematological parameters of all participants.

Parameter	HbA n=30 a	BPC n=30 b	STEADY n=30 c	p-value a vs b	p-value a vs c	p-value b vs c
Age (years)	31.50 \pm 8.25	29.23 \pm 9.21	27.7 \pm 8.56	ns	ns	ns
Gender: M/F	12/18	15/15	12/18	ns	ns	ns
Haematocrit (%)	38.77 \pm 4.31	23.92 \pm 5.07	23.83 \pm 4.32	0.000	0.000	0.859
WBC ($\times 10^3/\text{mm}^3$)	5.62 \pm 1.27	15.88 \pm 4.61	11.27 \pm 3.17	0.000	0.000	0.000
Platelets ($\times 10^3/\mu\text{L}$)	221.21 \pm 62.20	370.0 \pm 172.84	399.20 \pm 139.80	0.000	0.000	0.680

Data were presented as the mean \pm standard deviation (SD); ns not significant; Bold indicates statistically significant.

Table 2. Plasma levels of TNF- α , IL-8 and ET-1 levels in sickle cell anaemia patients in bone pain crisis (BPC), steady state (STEADY) and haemoglobin A controls (HbA). n=90.

Parameter	HbA n=30 a	BPC n=30 b	STEADY n=30 c	p-value a vs b	p-value a vs c	p-value b vs c
TNF- α (ng/L)	82.20 \pm 29.32	373.78 \pm 354.95	99.77 \pm 92.86	0.000	0.836	0.000
IL-8 (ng/L)	183.92 \pm 198.58	464.11 \pm 475.99	233.57 \pm 294.35	0.002	0.438	0.005
ET-1 (ng/L)	51.18 \pm 28.70	164.90 \pm 214.76	56.16 \pm 48.34	0.042	0.976	0.038

Table 3. Severity scores of sickle cell anaemia patients in a bone pain crisis.

Pain indices	Assessment	Frequency (%) n=30
Patients' perception	Verbal numerical	
	Pain score	
	Mild	2 (6.7)
	Moderate	13 (43.3)
Duration of pain	Severe	15 (50.0)
	1-4 days	15 (50)
	5-7 days	9 (30)
Doctors' perception of Patient's behaviour	More than 7days	6 (20)
	Normal	5 (16.7)
	Agitated	15(50.0)
	Very disturbed	8 (26.7)
Analgesia administered	Too quiet	2 (6.7)
	Paracetamol/NSAID	8(26.7)
	Opioids	22(73.3)
	PCA	0(0.0)
Hospitalized	ICU	0(0.0)
	Daycare	23 (76.7)
	Inward admission	7 (23.3)

NSAID Non-steroidal anti-inflammatory drugs; PCA Patient-controlled analgesia; ICU intensive care unit.

(20%) of the patients had pain for more than 7 days prior to presentation. From the physician's perception, five patients (16%) were normal in their behaviour, 15 (50%) were agitated, eight (26.7%) were very disturbed, and only two (6.7%) were too quiet during clinical examination. Most of the patients were relieved of pain following administration of opioids, 22 (73.3%); or paracetamol/NSAIDs, 8 (23.3%). None of the patients required patient-controlled analgesia or intensive care unit to relieve pain.

The perception of the patient and physician were summed up for ease of comparability as the total summary pain score (TSPS), **Table 4**. Most patients 15 (50%) had a moderate TSPS and 12 (40%) had a mild TSPS while three (10%) had severe TSPS.

Table 4. Total summary pain score (TSPS) of sickle cell anaemia patients in bone pain crisis.

Severity	TSPS	No of patients (%) n = 30
Mild	3-7	12 (40)
Moderate	8-11	15 (50)
Severe	12-16	03 (10)

TSPS = (PS x DP) +PB + AA; TSPS total summary pain score; PS Pain score based on patient's verbal, numerical score 0-10; DP Duration of Pain before presentation; PB Patient's Behaviour during an examination at presentation; AA- Analgesic administered to relieve pain; SCA sickle cell anaemia.

Site of bone pain crisis in the BPC group. Concerning the site of bone pain, multiple sites were involved. SCA patients in bone pain crisis presented with pain in the various parts of the body: The upper limbs were the most frequently involved parts with right being 13/76 (17%) and Left 17/76 (22%). The other sites involved were right lower limb 9/76 (12%); left lower limb 21/76 (16%); spine 12/76 (16%); and chest (ribs and/or sternum) 09/76 (12%).

Comparing the plasma levels of TNF- α , IL-8 and ET-1 in the bone pain severity groups. Plasma levels of the pro-inflammatory markers (TNF- α , IL-8 and ET-1) were compared among the BPC severity groups and shown in **Figures 1A-C**. Plasma levels of the pro-inflammatory mediators were highly variable. The severe BPC group had the most elevated mean plasma TNF- α (610.49 \pm 352.82 ng/L), IL-8 (691.61 \pm 966.20 ng/L), and ET-1 (336.40 \pm 24.38 ng/L). Mean plasma TNF- α level was significantly higher in the mild, moderate and severe than steady state group (p=0.000, p=0.004 and p=0.001 respectively), **figure 1A**. The IL-8 level was significantly higher in the mild and moderate groups than the steady state group (p=0.037 and p=0.019), **figure 1B**. Plasma ET-1 level was significantly higher in the mild and severe groups than the steady state group (p=0.013 and p=0.006), **figure 1C**. Post hoc (i.e. within the group) comparison of plasma level of TNF- α , IL-8 and ET-1 between mild and moderate; moderate and severe; and mild and severe bone pain severity groups were not statistically significant.

Correlation of TNF- α , IL-8 and ET-1 in sickle cell anaemia patients during bone pain crisis. The correlation was performed for TNF- α and ET-1 in SCA patients during bone pain crisis (n=30), **Figure 2**. Spearman rho correlation analysis among the SCA patients in the bone pain crisis group revealed a significant positive correlation (r=0.854, p=0.000) for TNF- α and ET-1. IL-8 and ET-1 were not significantly correlated (r=0.017, p=0.927) in the BPC group. Similarly, IL-8 and TNF- α (r=0.004, p=0.985) were not significantly correlated.

Discussion. The findings in the present study are similar to those of other studies, who found that

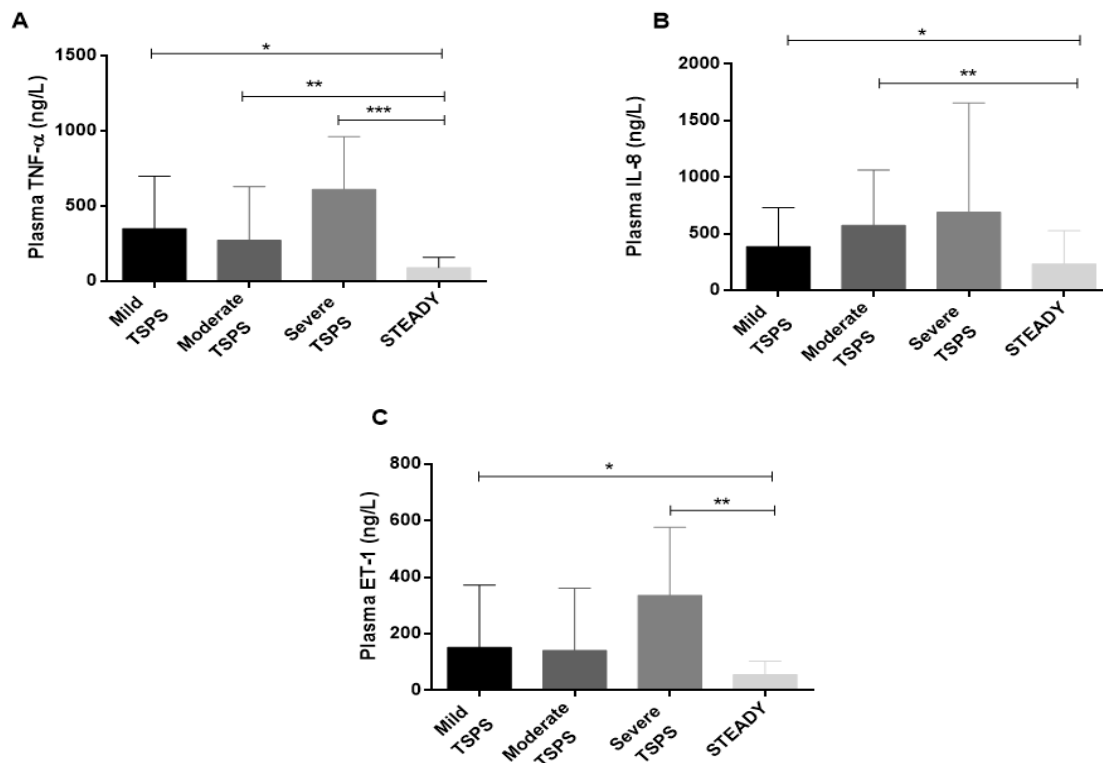


Figure 1. Plasma levels of TNF- α , IL-8 and ET-1 in SCA patients during bone pain crisis. **A:** Total summary pain score (TSPS) against plasma level of TNF- α in SCA patients with bone pain crisis. n=60. *p=0.000; ** p=0.004; *** p=0.001. **B:** Total summary pain score against plasma level of IL-8 in SCA patients with bone pain crisis. n=60. *p=0.037; **p=0.019. **C:** Total summary pain score against plasma level of ET-1 in SCA patients with bone pain crisis. Results are in mean \pm standard deviation. n=60. *p=0.013; ** p=0.006; STEADY - Steady state group.

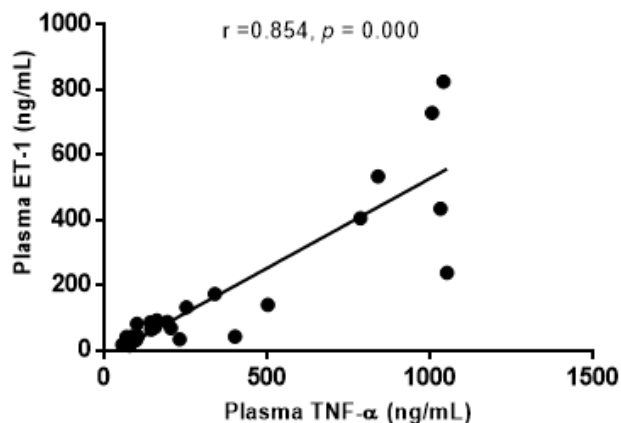


Figure 2. Correlation of plasma TNF- α and ET-1 in SCA patients during BPC. n=30.

levels of pro-inflammatory mediators were higher in SCA patients than in controls. Contrary to the study by Graido-Gonzalez et al. who found only ET-1 to be significantly higher in SCD patients before VOC than after crisis, the index study found that the three peptides studied were elevated in the BPC group.²¹ The smaller sample size and short post-crisis period of 1-3weeks may have accounted for the lack of significant difference in

the previously referenced study. Elevated levels of cytokines observed may be due to increased secretion by the leucocytes and platelets that were also significantly elevated in the SCA patients especially during BPC compared to controls.²⁸ IL-8 is known to be a chemotactic factor for neutrophils. Activation of neutrophils seen in SCA patients during VOC is believed to be mediated by IL-8 and augmented by other pro-inflammatory mediators.^{18,38} Elevated levels of the IL-8 in patients with SCA agrees with those of other researchers who found higher plasma IL-8 level during acute chest syndrome and in patients with vaso-occlusion.^{15,21} IL-8 propagate inflammation by increasing the adherence of sickle red cells to endothelium via the $\alpha 4\beta 1$ integrin receptors on sickle reticulocytes.^{25,39} The active process of endothelial adhesion contributes to the passive mechanical obstruction that leads to vaso-occlusion in SCA.^{6,40}

Significantly elevated TNF- α observed in the SCA groups is similar to findings of Lanaro et al. and Goncalves et al. Both groups of researchers found that SCA patients have increased circulating TNF- α and IL-8 levels at steady state and during

the crisis.^{15,16} Apart from enhancing endothelial adhesiveness, TNF- α activates leucocytes and the coagulation cascade, leading to the elevation of plasma levels of acute-phase plasma proteins such as fibrinogen that aid erythrocyte adhesion to endothelium.^{41,42} These contribute to the development of vascular occlusion in SCA patients. Endothelin-1 is a potent vasoconstrictor of arterioles and post-capillary venules that contribute to vaso-occlusion, bone pain crisis, acute chest syndrome and nephropathy. ET-1 upregulates synthesis of adhesion molecules like ICAM -1, VCAM-1, and E-selectin in endothelial cells thereby participating in leucocyte adhesion at sites of inflammation.^{21,27,28,43} An elevated level of ET-1 results from low oxygen tension in SCA patients because hypoxia is a potent stimulus for the production and release of ET-1 by vascular endothelial cells.^{21,39}

Comparing the verbal pain numerical scoring system with the total severity score system (adopted in this study for ease of comparability of variables), only three (10%) of the bone pain crisis SCA group were in severe pain at presentation. It would then not be surprising that most of the patients 23(76.7%) were managed as outpatients and none had pain that was severe enough to warrant patient-controlled analgesia (PCA) or intensive care unit (ICU) admission. This finding is similar to the outcome of the study by Etienne-Julan et al., in which an episodic pain index was adopted for children with SCD.²⁵ From those above, it could be inferred that elevated levels of pro-inflammatory mediators contributed to the severity of BPC in the patients. Pain and the immune system influence each other in such a manner that makes it difficult to determine whether blocking nociception or reducing the production of these pro-inflammatory cytokines

would result in less severe pain.⁴⁴ Pro-inflammatory cytokines stimulate pain via the cyclooxygenase-1/prostaglandin E2 induction at the tissue injury site and the spinal cord thus, increasing neuronal sensitivity to pain stimuli. This mechanism corroborates the observation relating to the location of pain in the BPC group. In this study (data not shown) BPC involving the spinal vertebrae was more frequent among the moderate and the severe pain groups probably because of the proximity of the infarctive injury to the spinal cord.^{44,45} IL-8 like other chemokines are responsible primarily for migration of leucocytes to the sites of tissue injury or inflammation as seen in VOC. It also participates in synaptic transmission and formation of secondary messenger systems in neurons and glial cells, hence favouring nociception.⁴⁵ Tumour necrosis factor- α and ET-1 were mostly positively correlated with the bone-pain crisis severity groups indicating the role of both peptides in the pathogenesis of the BPC.

Conclusions. The persistently elevated levels of these pro-inflammatory cytokines have further confirmed that SCA is a chronic inflammatory state and contribute significantly to the pathogenesis and the severity of pain in SCA. Therapies that target these inflammatory peptides could help to ameliorate or forestall bone pain crisis in SCA.

Limitation of the study. The fact that many patients would have used some analgesia to relieve pain prior to presentation could have affected the assessment of severity score. This possible error was catered for by the bi-directional assessment of pain using the adapted total severity pain scoring system.

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