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CARDIOVASCULAR CHANGES IN SICKLE CELL CRISES AND IMPACT OF ARGININE SUPPLEMENTATION IN VASO-OCCLUSIVE CRISIS AND ACUTE CHEST SYNDROME

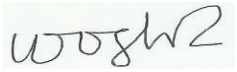
RICHARD ONALO

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, South Africa, 2020

DECLARATION

I, Richard Onalo declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any other University.



Signature of Candidate

___28th_ day of ___October___2020 in Johannesburg

DEDICATION

I dedicate this work to God the Almighty who made it possible for me to complete this work despite the mountain of challenges I had during the course of the work.

To my lovely wife and children, for their support and understanding throughout the period of my absence, in the pursuit of this thesis.

To my late friend, Dr Gcina Dumani, who took me in as a bosom friend while in South Africa.

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

1. **Richard Onalo,** Uche Nnebe-Agumadu, Oniyangi OO, Antoinette Cilliers, Peter Cooper. Effects of acute painful crisis on the blood pressures of children with sickle cell vaso-occlusive crisis. Oral presentation at the joint 14th triennial congress of Union of National African Paediatric Societies and Associations & 49th Annual General and Scientific Conference of Paediatric Association of Nigeria, 24th-26th January, 2018, Abuja, Nigeria.

2. **Richard Onalo,** Peter Cooper, Antoinette Cilliers, Uche Nnebe-Agumadu, Oniyangi OO, Oladimeji M. Damilare, Claudia R. Morris. Oral arginine therapy for children with sickle disease in Nigeria: The gains and challenges. Visiting Professor lecture at the Emory University School of Medicine, Atlanta, Georgia, USA, 5th December, 2019.

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2. **Richard Onalo,** Peter Cooper, Antoinette Cilliers, Uche Nnebe-Agumadu, Oniyangi OO, Oladimeji M. Damilare, Claudia R. Morris. Oral Arginine Therapy As a Novel Adjuvant in the Management of Acute Pain in Children with Sickle Cell Anaemia in Nigeria: *A Randomized Placebo-Controlled Trial. Blood 2019; 134(Supplement_1): 613.https://doi.org/10.1182/blood-2019-122510*

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ABSTRACT

Sickle cell disease, typified by sickle cell anaemia (SCA), is prevalent in Africa and is characterised by chronic anaemia and acute exacerbation of symptoms (crisis). The crisis state is fraught with complications in various systems, predominantly the cardiopulmonary system and the skeletal system. Nitric oxide (NO) deficiency has been implicated in some of the pathological changes observed in sickle cell crisis (SCA-VOE) and arginine, the obligate substrate for NO production, is thought to have a role in the management of SCA-VOE.

The study aimed to determine the influence of SCA-VOE on cardiovascular parameters and to explore the impact of arginine supplementation on the cardiac changes and the pain in children, 5-17 years, with crisis.

A comparative case-controlled design was used to determine the effects of crisis on the cardiovascular system while a double-blind, placebo-controlled randomised phase 2 trial (RCT) was designed to study the impact of oral arginine (100 mg/kg/dose q8 hours for five days) on the cardiac changes and the intensity of pain**.**

A total of 176 patients (92 in steady state and 84 in crisis) participated in the comparative case-controlled study, while 68 and 66 patients, participated in the RCT on the impact of oral arginine on cardiovascular changes and on the pain episodes in SCA-VOE, respectively.

The prevalence of tachycardia, hypertension, and conduction abnormalities were higher in SCA-VOE than in the steady state. Mean myocardial ischaemia score [3.14±1.61(crisis state) vs. 1.97±1.41 (steady state), $p<0.001$]; pulmonary artery systolic pressure [33.77±12.12 mmHg (crisis state) vs 22.71 mmHg (steady state), $p<0.001$], mean pulmonary artery pressure [25.00±7.78 mmHg (crisis state) vs. 18.67±5.16 mmHg (steady state), $p<0.001$] and median NT-proBNP [73.3 pg/ml (crisis state) vs. 6.00 pg/ml (steady state), $p = 0.001$] were higher in patients with crisis.

Arginine supplementation reduces mean pulmonary artery pressure, mean tricuspid regurgitation velocity, median troponin I and NT-proBNP levels, analgesic requirement, time-to-crisis resolution and length of hospital stay.

In conclusion, SCA-VOE is a stressor to the cardiovascular system and the administration of oral arginine during SCA-VOE reduces the prevalence of cardiac abnormalities and ensures faster analgesia, shorter time-to-crisis resolution and a shorter hospital stay.

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LIST OF ABBREVIATIONS AND SYMBOLS

ACS	Acute Chest Syndrome
AVCOT	Atrioventricular valve closure to opening time
ADMA	Asymmetric dimethyl arginine
AMAC	Abuja Municipal Area Council
BP	Blood Pressure
β -	Beta
CK-MB	Creatine kinase-myocardial isoenzyme
cm	centimetres
Cl^-	Chloride
CI	Confidence interval
CGRP	Calcitonin related gene peptide
CSSCD	Cooperative Study of Sickle Cell Disease
CW	Continuous Wave
DBP	Diastolic blood pressure
ED	Emergency Department
ECG	Electrocardiography
EF	Ejection fraction
ET	Ejection time
FCT	Federal Capital Territory
FS	Fractional shortening
Glu	Glutamine
gm	Grams

H^+	Hydrogen ion
HbS	Haemoglobin S
HbSS	Haemoglobin SS
HbS/β-thal	Haemoglobin S/β-thalassaemia
IVSd	Interventricular septal diameter at end-diastole
IVSs	Interventricular septal diameter at end-systole
IQR	Interquartile range
iNO	Inhaled nitric oxide
K^+ -	Potassium
KM	Michaelis constant
$_L$-Arg	$_L$-Arginine
LVIDd	Left ventricular internal diameter at end-diastole
LVIDs	left ventricular internal diameter at end-systole
LVOT	Left ventricular outflow tract
LV	Left Ventricle
LC-MS-MS	Liquid Chromatography-Mass spectrometry
LVPWs	Left ventricular posterior wall diameter at end systole
LVPWd	Left ventricular posterior wall diameter at end-diastole
MAPSE	Mitral annular plane systolic excursion index
MPI	Myocardial Performance Index
MI	Myocardial ischaemia
MQS	Medication Quantification scale
mmHg	millimetre mercury
MPAP	Mean Pulmonary Artery Pressure

MHz	megahertz
m/s	meter per second
μmol	micromole
ml	millilitres
ng	nanogram
NAFDAC	National Agency for Food and Drug Administration and Control
NSAID	Non-steroidal anti-inflammatory drug
NACSSG	National Acute Chest Syndrome Study Group
NT-proBNP	N-terminal prohormone brain natriuretic peptide
NO	Nitric oxide
PADD	Pulmonary artery diastolic pressure
PASP	Pulmonary artery systolic pressure
PCV	Packed Cell Volume
PAH	Pulmonary artery hypertension
PVR	Pulmonary vascular resistance
QTc	Corrected QT
RAD	Right axis deviation
RAP	Right atrial pressure
RBBB	Right bundle branch block
RBC	Red blood cell
RCT	Randomized control trial
Regurg	Regurgitation
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RV	Right ventricle

RVFAC	Right ventricular fractional area change
SBP	Systolic Blood Pressure
SCA	Sickle cell anaemia
SCA-VOE	Sickle cell anaemia vaso-occlusive episode
SCD	Sickle cell disease
SD	Standard deviation
SE	Standard error
SVR	Systemic vascular resistance
TAPSE	Tricuspid annular plane systolic excursion
TRV	Tricuspid regurgitation velocity
UATH	University of Abuja Teaching Hospital
VAL	Valine
VCAM	Vascular cell adhesion molecule
VcFc	Velocity of circumferential fibre shortening
VLA	Verylate activation antigen 4
VOC	Vaso-occlusive pain crisis
VTI	Velocity time integral
Ws	Wall stress
WHO	World Health Organization
Wu	Wood unit
δ	Sigma
<	Less than
>	Greater than
≥	Equal or greater than
%	Percent

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CHAPTER ONE

INTRODUCTION

1.1 General Introduction

The history of sickle cell disease (SCD), according to Ebrahim *et al*[1]and Desai *et al*[2]dates back to the 1870s when it was known as 'Ogbanje' in African medical literature meaning "children who come and go" because of the very high infant mortality rate associated with it. There are reports of SCD tracking back to 1670 in one Ghanaian family.[2] Although SCD primarily affects persons of African descent, it is also present in other races including Portuguese, Spanish, French Corsicans, Sardinians, Sicilians, mainland Italians, Greeks, Turks and Cypriots. It has also been reported in Asia, India and other Middle Eastern countries.[3]

The sickle cell gene reportedly arises from at least four independent mutational events, three originating in Africa and a fourth in either Saudi Arabia or central India. These independent events occurred between 3,000 and 6,000 generations ago.[4] It was in 1910 when a Chicago physician, James B. Herrick,[5] noted in a patient of his from the West Indies, an anaemia characterized by unusual red cells that were 'sickled shape.' In 1927, Hahn and Gillespie showed that sickling of the red cells was related to low oxygen.[6] Subsequent developments culminated in technological advancement in the management of the disease, and the approval of Food and Drug Administration of disease modifying agents such as hydroxyurea in 1998 and glutamine in 2017.[7] Though the care of children with sickle cell disease has improved remarkably, the disease remains one of the commonest causes of morbidity and mortality in sub-Saharan Africa.

Sickle cell disease (SCD) is an inherited disorder of the haematological system affecting about 250 million people globally.[4] The highest global prevalence of SCD is found in sub-Saharan Africa, South and Central America, Saudi Arabia and Mediterranean countries.[4]

Every year, approximately 300,000 live births worldwide are affected with this disorder, with 75% originating in sub-Saharan Africa and of these, 33 to 50% come from Nigeria.[8,9] This high incidence of a debilitating heritable disease has far reaching consequences on the wellbeing of the country's population. About 50-90% of children born with SCD die before adulthood and 70% of these deaths are preventable through low cost interventions.[10,11]

Sickle cell disease occurs when there is an inheritance of Hb S either homozygously (sickle cell anaemia, Hb SS), or in combination with other abnormal haemoglobins such as haemoglobin C (Hb SC disease), β-thalassaemia (Hb S/β-thal), haemoglobin O Arab (Hb SOArab) or haemoglobin D Punjab (Hb SD).[3] Sickle cell anaemia (SCA) is the commonest (consisting of 83% of all newborns with sickle cell disorders) and the severest form of sickle cell disease[3] and individuals with this condition tend to have more morbidity and are at higher risk of mortality.

1.2 Pathogenesis of organ injury in sickle cell disease

The molecular basis of sickle cell disease is the substitution of thymine for adenine in the sixth codon of the β-globin chain gene. The transcription message from this segment of the deoxyribonucleic acid is translated into valine instead of glutamic acid in the sixth position (β6Glu→Val).[12] Replacement of the hydrophilic glutamic acid by the hydrophobic valine predisposes haemoglobin S (HbS) to polymer formation especially when red blood cells containing HbS traverses the capillary microcirculation, Figure 1.1.[12] The polymer deformation of HbS triggers a cascade of other cellular changes including activation of the K+-Cl- co-transport system and the calcium gated potassium channel (Gardos channel) which results in cellular influx of calcium and efflux of potassium and water leading to cellular dehydration, rigidity of red cell membranes and increased red blood cell (RBC) fragility.[13-17] The increased fragility shortens the RBC lifespan from the normal 120 days to 10-20 days and is the major reason for the chronic anaemia and hence decreased oxygen-carrying capacity of blood. Hypoxaemia increases the propensity of sickled cells to adhere to vascular endothelium via interaction between very late activation antigen 4 (VLA 4) on the sickle cells and the vascular cell adhesion molecule-1 (VCAM -1) on vessel wall.

The changes in RBC rheology increase rouleaux formation and the tendency to intermittent microvascular obstruction and consequently ischaemic injuries to various organs.[13-17] Ischaemic-reperfusion injury is associated with increased production of reactive oxygen and nitrogen species (ROS, RNS respectively) through inhibition of mitochondrial electron chain activities and activation of several enzymes including inducible nitric oxide synthase, xanthine oxidase, cyclo-oxygenase and lipoxygenase, thereby creating a state of high oxidative load.[13-15]

Tissue damage has been documented in the spleen, kidneys, liver, brain, lungs, bones, muscles and retina.[14,15]

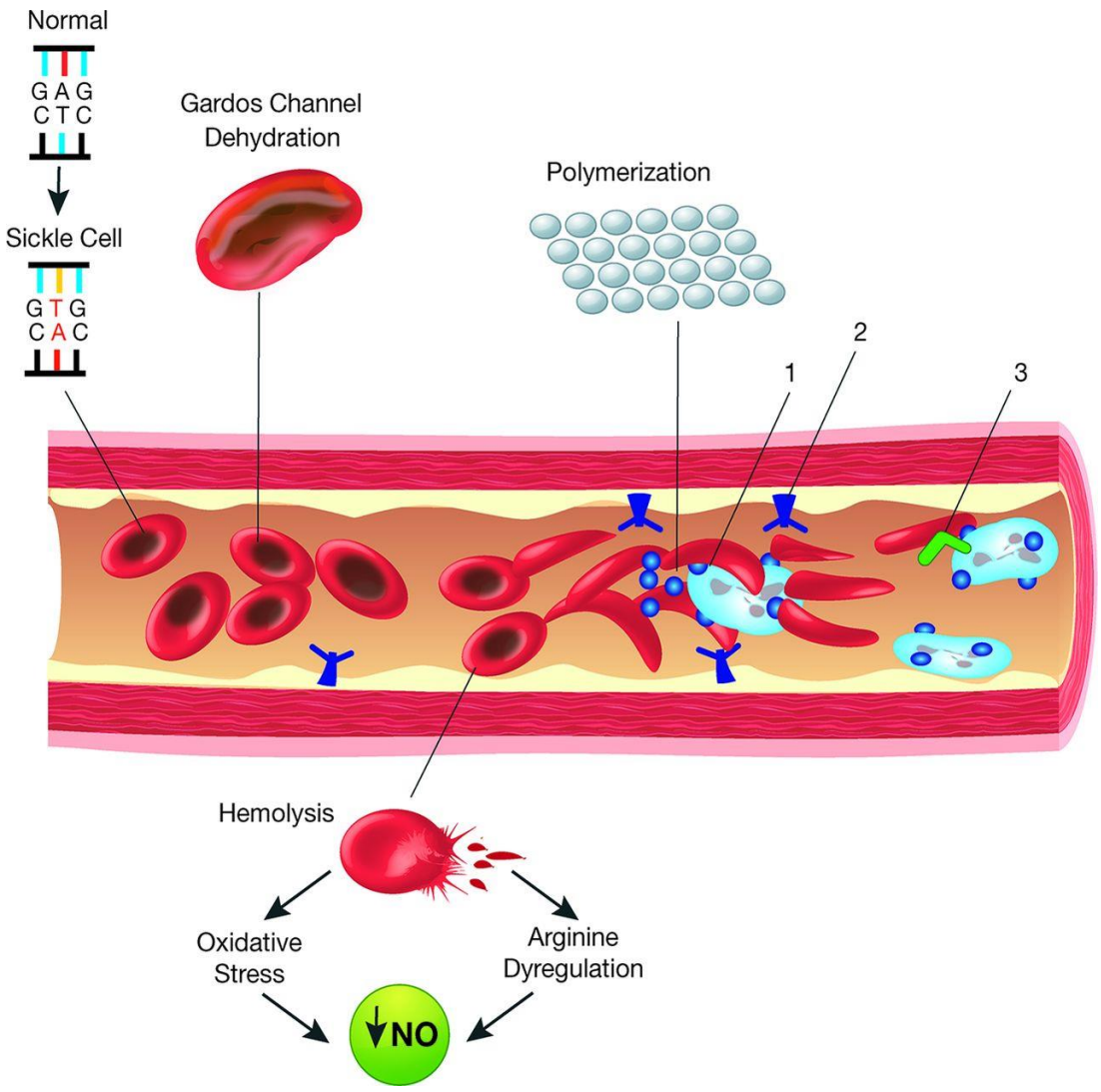


Figure 1.1: Simplified schematic representation of the pathophysiologic basis of sickle cell anaemia showing the single gene mutation, Gardos channel cellular dehydration and polymer formation. The mononuclear cells (labelled as 1), ligandin (labelled as 2) and receptors on mononuclear cells (labelled 3), contributes to the adhesiveness of the blood. Vascular obstruction, increased RBC haemolysis and arginine dysregulation contributes to the pathophysiology. Adapted from **Inusa et al, 2019**[12]

Organ injuries have been attributed to a high oxidative load, ischaemia, infarction, inflammation and hyperviscosity. Chronic oxidative stress plays a critical role in endothelial dysfunction, inflammation and multiple organ damage.[16,17]

1.3 Sickle cell crises

Sickle cell anaemia is characterized by chronic anaemia and acute episodic clinical events known as crises, which include vaso-occlusive (painful) crisis (VOC), acute chest syndrome (ACS), sequestration crisis, hyperhaemolytic crisis, central nervous system crisis (sickle cell cerebral syndrome), and aplastic crisis. The crisis may affect one organ or multiple organs systems.

Hyperhaemolytic crisis, aplastic crisis and sequestration crisis are associated with an acute precipitous drop in haemoglobin concentration on the background of chronic anaemia. The rapidity and severity of the drop may have a catastrophic effect on the child if not properly managed. In particular, SCA during both the steady state and in crisis, exerts a significant stress on the myocardium with varying degree of severity, ranging from subclinical ischaemia to frank cardiac failure. In addition to the effects on organs during sickle cell crisis, the patients experience excruciating pain that requires prompt and adequate management.

Although the precipitating factors for the occurrence of sickle cell crisis are not fully understood, infections, dehydration, hypoxia, extremes of temperatures, acidosis and stress are believed to play a major role either acting singly or in variable combinations.[18]

The frequency and severity of these crises have strong prognostic value. Those with frequent crisis are likely to die young from vital tissue damage. Sickle cell vaso-occlusion, which may involve the micro and macro vasculature, accounts for the majority of the sickle cell crises and explains most of its clinical manifestations.[18] Thus proficiency in management of vaso-occlusion in SCD is required to reduce the morbidity and mortality associated with SCD.

1.3.1 Vaso occlusive crisis

Pain is the hall mark of vaso-occlusive crisis and accounts for about 70% of hospitalizations in SCD patients.[19] The life of a SCD child is dominated by recurrent pain, most (90%) of which are managed at home. In a retrospective study[20] in Northern Nigeria, 987 patients presented with 2,652 episodes of VOC within a 6-year period. The sequence of pathophysiologic events leading to the perception of pain following vaso-occlusion in SCD is still not fully understood. It is however common opinion that tissue ischaemia from vascular occlusion results in

infarction and tissue damage which in turn initiates a cascade of secondary inflammatory response that interacts with neuroendocrine pathways and trigger release of norepinephrine that leads to increased sympathetic activity.[21] In the setting of tissue injury, norepinephrine release causes more tissue ischaemia, thus creating a vicious cycle.[21] It is the combination of ischaemic tissue damage and secondary inflammatory response that accounts for the acuteness and severity of SCD pain.

Pathophysiologically, tissue injury releases several major pain mediators such as interleukin-1, bradykinin, histamine, potassium ions, hydrogen ions, substance P and calcitonin gene related peptide (CGRP). Interleukin-1 leads to synthesis of prostaglandin E_2 and I_2.[21] Bradykinin, H^+, K^+, and histamine activate nociceptive afferent nerve fibers and evoke a pain response. Activated nociceptors then release stored substance P which facilitates the transmission of painful stimuli. Prostaglandins sensitize peripheral nerve endings and facilitate the transmission of painful stimuli to the cerebral cortex via A-δ and C fibers.[21] Vasodilatation with extravasation of fluids from the actions of bradykinin, substance P and CGRP leads to local tissue swelling and tenderness.

Sickle cell VOC pain often begins with swollen painful hands and feet in infancy but the site of pain gradually shifts to the arms, legs, back and pelvis as the child gets older. In the adolescent group, the pain often involves the chest and abdomen. Severity of pain varies from mild transient localized attacks of 5-10 minutes to severe generalized pain lasting for days and weeks and requiring hospital admission. The quality of the pain has been described by some patients as deep-seated. Severe bone pain crisis is usually characterized by extremely intense pain described to be worse than postoperative pain and is as intense as terminal cancer pain.[22] Severe acute VOC pain has been defined as pain requiring treatment in a medical facility with parenteral opioids for at least 4 hours.[22]

Despite the severity of SCA VOC pain, adequate analgesic cover is often not achieved due to under-estimations of the intensity of pain, and caregivers' preoccupation with the pathophysiologic causes of vaso-occlusion and fear of creating an opioid dependency state.[21,22] Patients are frustrated by the lack of attention commensurate to the intensity of pain and often report insensitivity of hospital staff, inadequate analgesic administration and overall lack of sympathy.[22]

Typically, the pain of VOC lasts 3-9 days. The clinical course of each pain episode has been categorized into an 8-phase phenomenon by Beyer *et al*[23] in 1999. In the first phase, the child has no pain but could identify the presence of factors that precipitated the VOC such as dehydration, changes in weather, etc. In the second phase, prodromal symptoms such as fatigue or jaundice appear. Pain appears in phase 3. It is initially mild but accelerates in phase 4 and peaks in phase 5. The pain may stay at this peak intensity for 48 to 72 hours. The pain begins to wane in intensity in phase 6 and steadily decline in phase 7 until it reaches the last phase. By phase 8, the pain is much more tolerable and the child is able to undertake normal activities. Most patients present to the emergency unit when they are in phase 5 and when home remedies have failed. It is therefore imperative to be able to provide adequate pain relief during their hospital admissions. Although most VOC episodes leave no disability after resolution of pain, frequent and severe VOC episodes are associated with a shortened lifespan.[21,22] Adequate pain management is therefore important in reducing morbidity and it hinges on an accurate assessment of the intensity of pain.

1.3.1.1 Pain assessment

A number of validated scales are available for assessment of intensity of pain. The scales include a visual analog scale, a verbal scale, a numerical scale and a Wong-Baker faces scale. It has been advocated that one uses a scale that is appropriate for the child's age. Once a pain assessment scale is chosen it should be persisted with so that the patient and caregiver become familiar with it.[21]

Initial pain assessment establishes a baseline against which subsequent assessments will be compared and analgesic effectiveness and dosing determined.

Difficulty in assessing pain in the paediatric population leads to inadequate pain management. Whilst self-reporting scales are useful in assessment of pain intensity in older children and adults, they cannot be used in infants and younger children without modification. The visual analog scale is a valid tool for assessing acute pain in children older than 7 years[22] and its use should be encouraged. The verbally administered numerical rating pain scale is the scale commonly used in hospitals and it has been validated for assessing pain in children. It is

recommended that pain levels should be routinely assessed during admission and that pain management should be a major component of the overall management of the patient.

1.3.1.2 Pain management

Adequate pain management is important partly because recurrent acute pain episodes may result in neurological changes that may heighten sensitivity to pain. In addition, severe painful episodes have been associated with development of acute chest syndrome and elevation of pulmonary artery pressures, both of which are risk factors for mortality. Although pain management is multidisciplinary, acute pharmacological intervention is the mainstay of therapy. Acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) are typically the first line therapy for mild pain but moderate and severe pains require a more potent analgesic such as opioids to abate. Combination therapy involving an opioid and NSAIDs has been shown to have synergistic analgesic effect in moderate and severe pain, although randomized controlled trials have failed to prove any advantage of combining opioids with the most potent parenteral NSAID (ketorolac) in the management of severe VOC pain.[22]

Opioids remain the drug of choice for quick, smooth and adequate control of severe VOC pain and should be used judiciously. It is well known that SCD patients clear morphine from their systems at a rate three times faster than non-SCD individuals and thus have greater tendency to develop tolerance,[22] hence, the need for increasingly higher doses of opioids to provide the same level of analgesia.

Opioid analgesics have fewer systemic adverse effects than NSAIDs and can be administered via several routes (intravenous, intramuscular, subcutaneous, and oral) but their use in SCD is associated with many myths about drug-seeking behaviour and addiction.[21] Once analgesic medications are commenced, patients should be monitored regularly to ensure the achievement of desired level of analgesia. Although pain relief can be assessed subjectively, pain management is clinically adjudged successful when there is a 1.5 cm sustained decrease in pain score from baseline on a 0-10 cm visual analog pain scale and the patient has transitioned to oral analgesic.[22]

Adjuvant therapies such as antihistamines, antidepressants, benzodiazepines, and anticonvulsants have been used to potentiate the analgesic effects of opioids. Various analgesic combinations have been used with no established permutations, thus making it difficult to effectively evaluate the superiority of one over the other. In addition, quantification of the total dose of analgesics required to achieve optimum pain control during an acute painful episode has been rendered cumbersome. A Medication Quantification Scale (MQS) tool[24-26] has been developed and validated for use in clinical and research settings to quantify and monitor pain medications and allows for the estimation of total analgesic dose used to achieve full analgesia from hospitalization to time of crisis resolution irrespective of the various analgesic combinations.

The MQS includes 10 classes of medications that are frequently prescribed for pain management: acetaminophen/aspirin, non-steroidal anti-inflammatory drugs, antidepressants, antihistamines, anticonvulsants, muscle relaxants, weak opioids, benzodiazepines, barbiturate/sedatives/hypnotics, and strong opioids. An MQS score is generated from the product of the detriment weight (defined as the negative impact each drug has in treating a given patient's pain) and the dosage level (determined by the recommended daily dosage). Though the MQS computation is cumbersome and time-consuming, it is an objective way of assessing the daily and total analgesics used in the treatment of a pain episode.

The conversion of opioids to oral morphine equivalent (equianalgesic dose) also provides a platform for computing the total opioid use and it allows for comparison of the opioid sparing capacity of a particular intervention over the other. Due to differences in drug potencies and duration of action, patients should not be changed from one opioid regimen to the other without recourse to their equianalgesic value.

A recent publication[27] suggests that arginine is a potential pain relieving adjuvant with the capacity to reduce the total opioid use for painful VOC episodes by at least 50%. Although the exact mechanism of arginine effect on VOC pain is not known there is scientific data suggesting that an increase in NO production, improved perfusion, reduction in microvascular vaso-occlusion and down-regulation of inflammatory pathways may be involved. The finding that arginine can be used as a pain relieving adjuvant is of great importance, especially in the milieu of growing concern of opioid addiction in sickle cell patients. Further studies are

needed to confirm the beneficial effects of arginine supplementation in management of VOC pain.

1.3.2 Acute Chest Syndrome

Acute Chest Syndrome (ACS) is the second most common reason for hospitalization in children with SCD. The syndrome occurs in about 48% of individuals with SCD and accounts for 25% of SCD-related mortality.[28-31] An incidence rate of 12.8-14 episodes per 100 patient-years was reported among homozygous SCD in the Cooperative Study of Sickle Cell Disease (CSSCD) report, while those with sickle cell/β^+ thalassemia and sickle haemoglobin C disease had an incidence of 9.4/100 patient-year and 5.2 cases/100 patient-year, respectively.[29] In another study,[30] the National Acute Chest Syndrome Study Group (NACSSG), most SCD patients that were hospitalized for vaso-occlusive pain crisis went on to develop ACS later in their hospital course. Development of ACS prolongs duration of hospital stay. The mean duration of hospitalization ranged from 6.4 to 10.5 days in the CSSCD report.[31]

The association of severe VOC with development of ACS has been established in adults.[30] VOC occurred shortly before severe ACS in 40% of patients and was present in 80% of adults with ACS. In a recent study in children, ACS occurred 24-72 hours following hospitalization for VOC.[32] In another study involving 120 children hospitalized for sickle cell crisis in Northern Nigeria, 80 (66%) had ACS and 66 (82.5%) of these had associated VOC.[33] During VOC, bone ischaemia may lead to infarction, necrosis of bone marrow and subsequent release of marrow contents into the blood circulation, some of which may gain access to the pulmonary circulation and cause pulmonary fat embolism. In addition to embolization, thoracic bone infarction may contribute to ACS by splinting the rib cage, enhancing atelectasis, promoting ineffective clearance of secretion and consequent pulmonary infection. The role of thoracic bone infarct in ACS was brought to the fore by Bellet *et al*,[34] when the authors discovered that about 40% of sickle cell patients hospitalized for ACS had thoracic bone infarction.

Diagnosis of ACS is clinical and requires the appearance of a new infiltrate on chest radiograph in combination with one or more symptoms and signs such as chest pain, cough, wheezing, tachypnea and fever in an individual with SCD. The haematologic response to ACS is remarkable. The haemoglobin (Hb) concentration decreases by 0.7 g/dl while the white blood

cell count increases by 70%.[31] These haematological changes may be accompanied by metabolic derangements such as an elevated lactate dehydrogenase, rising serum bilirubin levels, and hypoxemia, thus justifying the need for frequent or continuous pulse oximetry and biochemical monitoring.

Currently the management of ACS revolves around adequate supportive care (good hydration, oxygen therapy and optimum pain control), adequate antibiotics cover, blood transfusion and chest physiotherapy. Newer and experimental therapies[35,36] are emerging in the literature and these include anticoagulation with tinzaparin, inhaled nitric oxide (iNO) therapy, and arginine therapy. The role of arginine as the sole source of nitric oxide has been the focus of scientific evaluation in the recent times and there are indications that this amino acid will improve the management of VOC and ACS in the future.

The effects of severe VOC and ACS on a child already disadvantaged by SCD are numerous and may include physical disability from organ changes, hospital confinement and loss of school hours. Incidentally, VOC and ACS are the most common causes of morbidity in children with SCD. Discovery of a nutritional supplement that will ameliorate the biochemical changes and hence the manifestation of the crisis may positively alter the natural course of VOC and ACS. Organ changes in VOC and ACS may be clinical or sub-clinical, benign or extremely life threatening. The life threatening sequelae of VOC and ACS affects the cardiopulmonary system mainly and may include acute increases in pulmonary pressures, myocardial ischaemia, and myocardial dysfunction. Therefore, meticulous attention to the cardiac and pulmonary effects is necessary to reduce the mortality associated with severe ACS and VOC.

1.4 The heart in sickle cell disease

Cardiac disorders are frequently described in patients with SCD.[37-39] The prevalence of cardiac abnormalities detected using echocardiography in children has been reported to be as high as 83% of patients in some parts of Africa.[37,38]

1.4.1 Cardiac structures and functions in steady state

1.4.1.1 Structural abnormalities

Various structural abnormalities have been documented in children with SCD. Findings may include dilated cardiac chambers, ventricular hypertrophy and increased ventricular mass. The structural changes are age-dependent and are commonly related to the degree of anaemia. The changes in cardiac structures may begin before the age of one year especially in children with severe forms of SCD and progress with age such that by the age of 5 years, most children with SCD have developed cardiomegaly. Cardiomegaly was documented in more than half of 289 children with sickle cell anaemia in a Sudanese study, especially amongst school age children.[38] Elevated cardiac output due to increased stroke volume is also a common finding.[39] The increased cardiac output serves as a compensatory response to maintain tissue oxygenation under conditions of chronic anaemia. The cardiac chambers enlarge to accommodate the high blood volume which in turn results in progressive cardiomegaly. Ultimately the progressive dilatation of the ventricles results in increased wall stress which in turn induces myofibril elongation, hypertrophy and eventually increased left ventricular mass. Lester *et al*[39] evaluated 64 children with sickle cell disease and found a significantly larger left ventricular mass of 120.4±3.24g compared to 89.9±1.81g in a normal control group ($p<0.001$).

1.4.1.2 Systolic and diastolic dysfunction

In the late 1990s, San *et al*[40] documented left ventricular systolic and diastolic dysfunction in 54 adults aged 22.6±7.3 years with SCD but subsequent authors were unable to consistently demonstrate myocardial dysfunction.[41,42] Just as in the adults, studies in children showed no uniformity in their results. There is variable reporting of diastolic and systolic dysfunction of the left ventricle[37,43] with some authors documenting diastolic dysfunction associated with normal systolic function[44,45] and others reporting normal systolic and diastolic function.[46] Nabur *et al*[37] for example found systolic dysfunction in 23.3% and diastolic dysfunction in 51% of 99 children with SCD in Tanzania, while Lamers *et al*[47] reported a lower but normal percentage fractional shortening and velocity of circumferential fibre shortening as well as contractility index among 57 SCD children between the ages of 3 months and 18 years.

Some of these findings suggest the probable presence of sub-clinical systolic dysfunction in SCD, particularly amongst individuals with the severe form of the disease. A few authors[39] have argued that 'normal' systolic function in SCD, in fact, may actually be a reflection of suboptimal ventricular function because these patients are actually expected to demonstrate increased systolic function in response to the increased preload and the decreased afterload that characterize the disease.

The use of conventional load-dependent indices of cardiac function such as systolic time intervals, velocity of circumferential fibre shortening (VcFc), fraction shortening (FS) and ejection fraction (EF) in children with SCD may show false negative results. Several publications[43,47,48] however, have indicated that the use of nongeometrical index of cardiac function (the 'myocardial performance index' or Tei index) and 'rate-corrected velocity of circumferential fibre shortening-wall stress (VcFc-Ws) index' tended to report values suggestive of systolic and diastolic cardiac dysfunction.

Data pertaining to diastolic performance of the left ventricle in sickle cell disease is more certain. Echocardiographic measures of diastolic function such as E-wave deceleration time, early mitral inflow/annular movement ratio and mitral E-wave to A-wave velocity ratio as well as abnormal Tei index in the milieu of normal indices of systolic function have been reported to be higher in SCD subjects.[48,49] The measurements usually have a negative correlation with haemoglobin concentrations suggesting a relationship between sickle cell disease severity and the degree of diastolic dysfunction. Diastolic dysfunction is considered to be an early marker of cardiac involvement in SCA and may progress to overt congestive heart failure with systolic dysfunction if not treated early.[49]

1.4.1.3 Pulmonary hypertension

The presence of pulmonary hypertension is suspected by the documentation of a tricuspid regurgitation jet velocity (TRV) of ≥2.5 m/s. A TRV of ≥2.5 m/s has been reported in about 1/3 of adults with SCD.[50,51] Data on the prevalence and risk factors of pulmonary artery hypertension (PAH) in children with SCD are limited. The accepted echocardiographic definition of PAH is a pulmonary artery systolic pressure (PASP) of > 30 mmHg, which

corresponds to a peak tricuspid regurgitant velocity (TRV) of ≥2.5 m/s. The application of this definition in children with SCD has demonstrated a high prevalence of PAH, ranging between 11% and 46% in some series.[52-55] However, the association of elevated PASP or elevated TRV with the risk of mortality have not been established in children.

1.4.2 Cardiac effects of sickle cell crisis

Information abounds on the cardiovascular changes during the steady state. There is, however, paucity of data on the state of the heart during sickle cell crisis. During sickle cell crisis, the heart is subjected to stresses resulting from an increased oxidant burdens, nitric oxide depletion and possibly myocardial ischaemic insults. Attention to the cardiovascular system is of extreme importance during crisis as cardiopulmonary complications may underlie sudden and unexplained SCD-related deaths.[56]

1.4.2.1 Myocardial ischaemia

The sickling process that takes place in small vessels can occur in the pulmonary and myocardial microcirculation and result in myocardial ischaemic injury. Bode-Thomas *et al*[57] in a study on children with sickle cell anaemia, observed ECG changes consistent with myocardial ischaemia (MI) in 89.5% of patients during vaso-occlusive crises and in 72.5% of those in a steady state. In that study, the mean MI scores during vaso-occlusive crises were significantly higher than those obtained in a steady state (1.84±0.20 versus 1.15±0.15, p = 0.017). Furthermore, children with VOC had a 3 times more chance of developing MI than those in a steady state.[57] This position was corroborated by the report of Norris *et al*[58] that demonstrated evidence of MI in 80% of adult SCA in crisis. A more elaborate study by de Montalembert *et al*[59] reported myocardial perfusion defects in 14 (63.6%) of 22 SCA children with chest pain and electrocardiographic evidence of myocardial ischaemia.

Diagnosis of MI during severe VOC and ACS requires a high index of suspicion because the dramatic presentation of VOC and ACS has the tendency of drawing attention to the non-cardiac pains originating from rib infarction, thoracic muscles, and even distant bone pains at the expense of the co-existing cardiac pain. An autopsy report[60] showed coronary artery occlusion by sickled RBCs in a patient that suffered a sudden death. Conduction abnormalities

resulting from the myocardial ischaemia has been postulated to be the cause of sudden death in this category of patients.[56]

Diagnosis of MI should be made using WHO criteria which combine molecular markers of myocardial injury with clinical and electrocardiographic features consistent with MI such as ST-T wave changes and QTC dispersion. Although the use of ECG alone is often not helpful, the scoring system developed by Bode-Thomas *et al*[57] using combinations of ECG findings appears to be sensitive in identifying children with MI. Cardiac biomarkers such as creatine kinase-myocardial isoenzyme (CK-MB) and troponins (C, T, I) are elevated in MI. However, creatine kinase is not specific and may also be released from damaged skeletal muscles during VOC. The troponins are more specific to myocardial injury and are a more accurate tool in identifying individuals with MI. Normal levels of cardiac troponin in the blood are very low and sometimes undetectable, but they rise sharply and quickly in response to a heart muscle injury and are therefore valuable at detecting MI early, even if very mild.[61] In a case-controlled study involving 26 SCD children with VOC and 27 SCD children in steady state (controls), Adegoke *et al*[61] demonstrated a significantly higher mean serum troponin T in those with VOC than in the control: 119±24.40 ng/L versus 57.90±13.80 ng/L (p=0.001). In that study, serum troponin T levels had a strong positive correlation with the pain severity scores (r =0.64, p=0.011). It may be concluded that an investigative protocol that combines ECG MI score with cardiac biomarkers should be able to identify majority of SCD patients with MI during a crisis.

1.4.2.2 Acute increases in pulmonary hypertension

Vaso-occlusive pain crises are often associated with worsening anaemia, enhanced red cell adhesion, hypoxemia and vasoconstriction which could promote or exacerbate pulmonary hypertension. Acute increases in pulmonary pressures during crises in SCD patients who already have severe anaemia and mild (to moderate) pulmonary hypertension may result in cardiovascular collapse.[62] It has been suggested that SCD patients are not able to tolerate even a slight increase in pulmonary artery pressure because they are already living at the limits of maximum cardiopulmonary compensation.[52] In addition, an increased cardiac output is required during VOC in patients that are already surviving in a state of high cardiac output demand.

Elevations in pulmonary pressures is common during ACS and up to 13% of affected individuals may develop cor pulmonale.[63] Another study by Machado *et al*[52] has also shown that pulmonary pressures are elevated during VOC. Studies on echocardiographic markers of pulmonary hypertension, particularly tricuspid regurgitation velocity (TRV) have shown an elevated TRV (defined as TRV ≥ 2.5 m/s) in 21.6% of children with SCD living in their first decade of life[64] while in another study involving children and adolescents with SCA, aged 8-24 years, 46.2% had TRV ≥ 2.5 m/s, among whom 11.5% had TRV ≥ 3.0 m/s.[65] Although elevated TRV is not synonymous with pulmonary hypertension, TRV has been validated as a useful screening tool for pulmonary hypertension,[62,65] but then, complete TRV envelope may not be present in some patients with elevated pulmonary pressure, and even when present, elevated right ventricular pressure may limit the extent of the tricuspid regurgitant jet which may result in underestimation of the pulmonary artery pressure. In addition, the presence of pulmonary valve stenosis and/or right ventricular systolic dysfunction, may limit the accuracy of pulmonary artery systolic pressure estimated from TRV.[66] The use of RV-focused apical four chamber view, frame rate ≥20 Hz with colour Doppler and intravenous agitated saline may surmount the challenges associated with acquisition of complete TRV envelope.[66]

A TRV jet of ≥ 2.5 m/s is assumed to correspond to a systolic pulmonary artery pressure of 30 mmHg or more and this has been used for research purposes to define elevated pulmonary artery pressure in adults with sickle cell disease.

During crisis, TRV has been shown to increase from a mean of 2.4 ± 0.07 m/s in steady state to 2.9± 0.07 m/s during VOC ($P < 0.001$) in an adult study from the USA.[62] In another study, the prevalence of TRV ≥2.5 m/s rose from 32% in steady state to 60% during crisis.[63] There was a mean pressure increase in excess of 96% between the mean pressures at steady state (20-40 mmHg) and during ACS (38-67 mmHg) in that study. An increase in TRV may be related to increases in serum endothelin-1, a usual accompaniment of VOC. Although similar studies are rare in children, a similar response could be expected in children with acute VOC and ACS. The increase in right ventricular afterload increases right ventricular end-systolic pressure which is transmitted through to the right atrium resulting in an increased TRV. The elevated TRV has been shown to have positive correlation with brain natriuretic peptide ($p<0.01$) and cardiac troponin I ($p<0.01$).[63] Thus, the combination of an elevated TRV with plasma N-terminal prohormone brain natriuretic peptide (NT-proBNP) level is likely to identify children

with pulmonary hypertension. In a cohort of 43 children with sickle cell disease, Takatsuki *et al*[67] demonstrated a positive correlation between NT-proBNP and TRV, r = 0.46, p <0.05. In another study involving non sickle cell patients with pulmonary hypertension, NT-proBNP showed a 100% sensitivity and 94% specificity for detecting right ventricular systolic dysfunction.[68] Although, many cardiopulmonary pathologies like left ventricular hypertrophy or dysfunction, acute coronary syndrome, pulmonary embolism and hypertension can present with elevated natriuretic peptides, several pulmonary hypertension risk stratification tools included NT-proBNP as a critical component for the diagnosis and stratification of patients with pulmonary artery hypertension,[69] and in some centres, NT-proBNP point-of-care devices have been introduced to shorten the time taken to make diagnosis of pulmonary hypertension. [69]

1.4.2.3 Increases in myocardial wall stress

The hyperkinetic circulation associated with chronic anaemia in sickle cell disease results in an eccentric ventricular hypertrophy and cardiomegaly, and cardiac chamber enlargement progresses with worsening anaemia.[70,71] Despite myocardial remodelling, individuals with SCD have increased myocardial wall stress. Apart from volume overload in SCD, acute increases in pulmonary pressures during crisis may create additional strain on the heart and hence increase in ventricular wall stress. Studies on effect of sickle cell crisis on myocardial wall stress are rare, probably due to the fact that the method involved in determining wall stress index is cumbersome.[72] Furthermore, wall stress as calculated based on Laplace formula may misrepresent afterload in children and young adults.[72] These pitfalls may have contributed to the declining use of wall stress index in many studies. Additional information in this regard is therefore needed.

1.4.2.4 Decreases in ventricular function

Worsening of anaemia is a frequent complication of VOC and ACS. Some authors[31,73,74] have documented an acute fall in haemoglobin concentration ranging between 1g/dl and 2.2 g/dl in children and adults with VOC and ACS. Such acute decreases could further worsen the oxygen-carrying capacity of blood and impact negatively on ventricular performance in a heart already functioning in a maximally compensated state. In a review of 113 SCA patients that

had blood transfusions in Southern Nigeria, 85.4% were admitted primarily for VOC but 24% progressed to develop anaemic cardiac failure.[75] Thus, ventricular dysfunction partly due to worsening anaemia, increased pulmonary pressures and myocardial ischaemia should be excluded in children with severe VOC and ACS.

1.5 Role of arginine in sickle cell disease

Management of sickle cell crises for decades has hinged on control of symptoms and prevention of complications. Treatment of painful vaso-occlusive episodes includes adequate analgesia through judicious use of parenteral opioids and/or nonsteroidal anti-inflammatory drugs, and hydration. Additional supportive therapies such as bedrest, application of heat, and massage of pain sites are also used in the management of SCD-VOC. Medications targeting the underlying mechanism of sickle-related pain are lacking. More recently however, reports of randomized controlled trials of therapies that target the mechanism of acute vaso-occlusive pain have started to emerge in scientific conferences and publications and include the use of rivipansel (GMI-1070),[76] intravenous magnesium,[77] polaxamer-188,[78] lidocaine,[79] inhaled nitric oxide,[80] low-molecular-weight heparin,[81] and arginine[27] as adjuvant therapy in pain management. Although, many of these studies indicated promising results, they were found on close scrutiny to have weak clinical outcomes and/or poor scientific methodologies. The study on arginine however, appeared to have a more convincing evidence with clearly demonstrated opioid-sparing effects and a trend to shorter hospital stay in children hospitalized for severe VOC. This evidence makes further study on the role of arginine therapy in sickle cell crises appealing.

L-arginine is a semi-essential amino acid involved in many metabolic pathways in the body. Although it can be synthesized in adequate amounts *de* novo, requirements may increase under conditions of increased stress and catabolism such as trauma, burns, sepsis and SCD crisis. During an episode of vaso-occlusive crisis, increased nitric oxide (NO) consumption increases arginine metabolism, Figure 1.2.

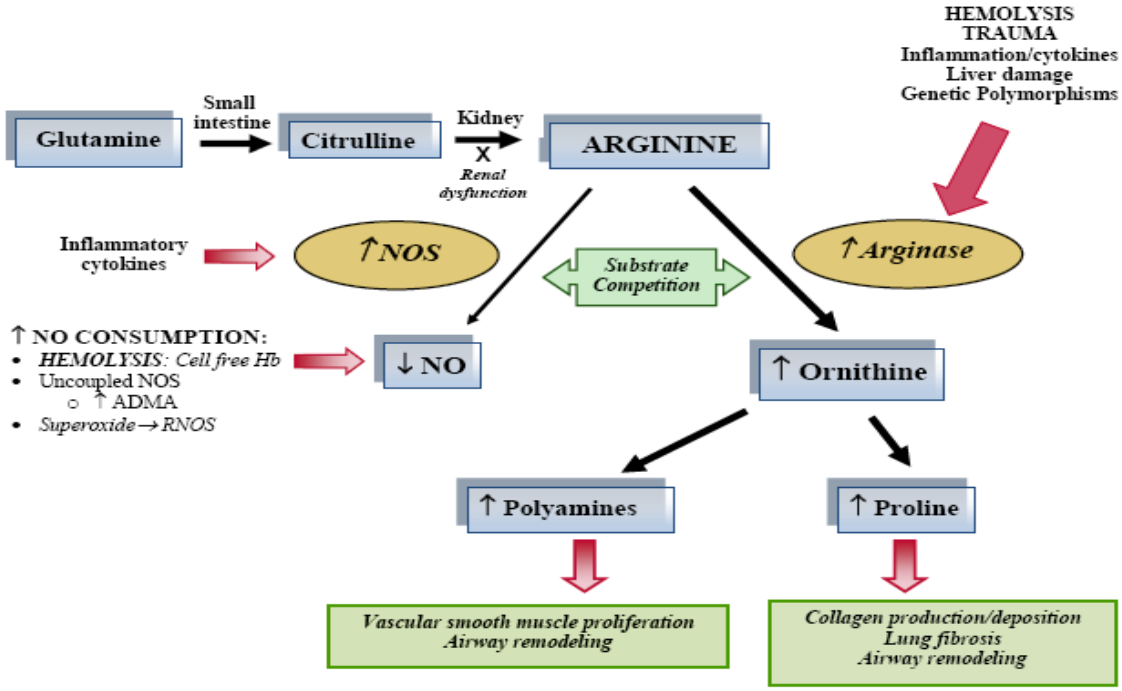


Figure 1.2: Schema demonstrating pattern of altered arginine metabolism in sickle cell disease. Adapted from Morris 2008 (American Society of Hematology Education Program, 2008)[82]

Arginine also serves as the substrate for synthesis of protein, including polyamines, proline, glutamate, creatine and agmatine. Each of these byproducts of arginine metabolism may participate in other metabolic pathways and cumulatively, may contribute to the relevance of arginine in many physiological and pathological states. In 2014, Rizk *et al*[83] indicated that $_{L}$-arginine induces reduction in myocardial fibrosis in diabetic Wistar rats, a property that may be of interest in the management of some sickle cell disease individuals that may be at risk of cardiac fibrosis from repeated episodes of myocardial ischaemia. However, another study by Kralova *et al*[84] failed to show effect of arginine on cardiac fibrosis induced by isoproterenol in rats. These conflicting reports suggest the need for further studies on the clinical relevance of the arginase pathway in arginine metabolism.

The nitric oxide (NO) synthase pathway, on the other hand, utilizes $_{L}$-arginine as an obligate substrate for the production of nitric oxide, an endogenous regulator of vasculature that promotes vasodilatation and inhibits vascular smooth muscle proliferation. NO also affects leukocyte adhesion, platelet aggregation and controls vascular oxidative stress, all of which are

implicated in the pathogenesis of VOC and vascular endothelial dysfunction. Haemoglobin released from lysed RBC rapidly scavenges NO at a rate 500-1000 fold faster than intra-erythrocyte Hb resulting in nitric oxide depletion, increased platelet aggregation and adhesion, and catabolism of arginine.[62] Studies have shown that children with SCD have low plasma arginine levels especially during a crisis.[82,85-87] Plasma arginine concentrations may be as low as 40-50 µmol/l, which is below the half-saturation constant (K_m) for the arginine transport system (approximately 100 µmol/l).[88] Any further reduction in plasma arginine concentration can adversely affect arginine uptake and bioavailability.

A reduction in plasma arginine levels has been shown to be associated with endothelial damage, multiple organ injury, increased haemolysis, pulmonary hypertension and increased mortality.[87,88] Under conditions of low arginine concentration, NO synthase is uncoupled, producing reactive oxygen species in lieu of NO, which further enhances oxidative stress and increases tissue injury.[88]

Arginase has also been shown to be released from lysed RBCs, resulting in elevation of serum arginase levels. Vilas-Boas *et al*[89] have demonstrated an almost two-fold increase in serum arginase level (median 21.1 ng/ml versus 14.7 ng/ml in normal non sickle cell individuals) in 50 adults with SCA in a steady state. This high level of arginase readily converts $_L$-arginine into ornithine and urea, thereby further reducing arginine bioavailability.

Low plasma arginine is predictive of the need for hospital admissions in children with SCD presenting to the emergency care unit,[90] while the ratio of arginine-to-ornithine and global arginine bioavailability ratio (arginine/ornithine+citruline) had a negative correlation with mortality in SCA children in a report from Dar-es-Salaam, Tanzania.[87] Mean plasma arginine values and arginine/ornithine ratio in healthy non-SCD children in parts of Africa are 120±10 µmol/l and 1.35±0.49, respectively compared to arginine levels of 34±15 µmol/l and a global arginine bioavailability of 0.37±0.10 in SCA children with fatal outcomes.[87]

1.5.1 Pharmacokinetics and safety profile of $_L$-arginine

Data on the clinical pharmacokinetics of $_L$-arginine in SCD patients are rare. Much of the information on the handling of $_L$-arginine in the body is provided by the work of Bode-Böger *et al*[91] in healthy volunteers. Peak plasma concentrations of 800 µmol and 6200 µmol were

achieved within 20-30 minutes of intravenous administration of 6g and 30g, respectively. While oral administration of 6g of L-arginine yielded a peak plasma concentration of 300 µmol at 90 minutes.[91]

Orally administered L-arginine is rapidly and almost completely absorbed, but extensively metabolized by enterocytes, resulting in oral bioavailability of 68±9% (range, 51-87%).[91] The half-life of L-arginine following oral administration of 6g was 1.5-2 hours.[91]

In the paediatric population, research has been done by Morris *et al*[35,92] to establish the therapeutic dose and safety profile of L-arginine in SCD patients both in a steady state and during a crisis. In a study involving 6 SCD patients and 4 normal controls, administration of 100 mg/kg of oral L-arginine resulted in increase in plasma arginine levels from a mean of 45.5±10.6 µmol/l to 144.7±30.3 µmol/l at approximately 2 hours after arginine administration but with a paradoxical decrease in NO production. During VOC, however, the same dose induced a 109.4% increase in NO production. This response suggests that SCD patients are unlikely to benefit from supplemental arginine during steady state but will need exogenous administration of L-arginine during VOC to maintain NO synthesis. At lower doses (50 mg/kg), a paradoxical decrease of 47.7% in NO production was recorded, despite an increase in serum arginine during VOC.

In another study,[27] the authors demonstrated clinical safety with L-arginine at a 100 mg/kg/dose, 8 hourly in a placebo-controlled double blind study involving 36 SCD children with 56 episodes of VOC. No arginine-related adverse effects were seen after 5 days of supplementation. One patient on the arginine arm however, developed hives during arginine infusion but the reaction was later found to be due to an allergy to the paper tape used to secure the intravenous line.

- L-arginine has been recognized as one of the least toxic of all amino acids both in animal and human trials. Daily dietary arginine consumption is between 2 and 7 grams,[93] commonly from nuts (like walnuts, hazelnuts, peanuts, almonds, cashews), seeds (like sesame and sunflower), dairy products, and meat. Studies have shown that higher doses, even as high as 30-60 grams per day, are well tolerated but because of the large number of pills required to be taken, intravenous infusions were preferred in some studies.[93] It is noteworthy that gastrointestinal

symptoms such as nausea, dyspepsia, vomiting, diarrhoea and a bad taste have been associated with long-term oral administration of arginine.[93]

1.5.2 Clinical experience with arginine therapy in sickle cell disease

Clinical trials in humans were based on results of studies with transgenic mouse models of SCD and the documentation of alteration in arginine metabolome in adults and children with SCD. L-Arginine supplementation in transgenic mice caused inhibition of the Gardos channel[94] and reduction in red blood cell density.[94] These actions resulted in improvement in organ perfusion and reduction in lung injuries as well as decreases in microvascular occlusion and death.[95,96] In addition, studies[97,98] have indicated the potential usefulness of arginine in increasing the antioxidant property of red blood cells, a much needed action in the milieu of high oxidative stress often encountered in patients with sickle cell crisis.

1.5.2.1 Refractory leg ulcers

Reports indicating the ability of arginine to induce healing of recalcitrant leg ulcers in patients with SCD appeared in the scientific literature in the 1990's.[98] Subsequent reports by other authors[99,100] corroborated this earlier finding. The report of Morris *et al*[35] confirmed the role of L-arginine in controlling chronic leg ulcers as well as in reducing the tricuspid regurgitation velocity in patients with pulmonary hypertension. In that report, a short 5-day course of arginine therapy was enough to result in wound healing. Although the sample size was small, the potential therapeutic role of L-arginine in the management of chronic leg ulcers was demonstrated. Furthermore, a randomized controlled trial[100] has showed satisfactory wound healing in 78% of patients with chronic leg ulcers who had L-arginine along with conventional wound care compared to 28% success rate in patients that had only conventional wound care ($p<0.001$). These reports suggest that both oral and parenteral L-arginine therapy play significant role in the control of leg ulcers complicating SCD.

1.5.2.2 Pulmonary hypertension

Other research[86,88] suggest that elevated pulmonary pressures in SCD are associated in part with dysregulated arginine metabolism and decreased arginine bioavailability. Clinical trials in

humans have demonstrated the therapeutic benefits of supplemental arginine in individuals with idiopathic and secondary pulmonary hypertension.[101,102] Mehta *et al*[102] found a 27.6% reduction in pulmonary vascular resistance and a 16% reduction in the mean pulmonary artery pressures in 10 adult patients with pulmonary hypertension (secondary to chronic ischaemic cardiomyopathy) following infusion of high dose arginine. The report of Mehta *et al*[102] was corroborated by another report that indicated improved oxygenation and decreased pulmonary vascular resistance in infants with persistent pulmonary hypertension of newborn.[103] The hypothesis of the usefulness of arginine was tested by Nagaya *et al*[101] in a placebo-controlled study involving 10 adults with pulmonary hypertension. These authors also found a significant reduction in mean pulmonary artery pressure and pulmonary vascular resistance.

A promising study by Morris *et al*[35] demonstrated a 15.2% reduction in mean pulmonary artery pressure with just 5 days' administration of oral $_{L}$-arginine in 10 patients with SCD. Although, the 15.2% reduction in mean pulmonary artery pressure appears small when extrapolated to the general paediatric population, but in the context of sickle cell patients, in whom a little increase in pulmonary pressure may be devastating,[52,62] such a degree of reduction in pulmonary artery pressure within five days of arginine therapy may confer some clinical advantage. Thus, with the increasing recognition of the contribution of pulmonary hypertension to the morbidity and mortality in SCD, the need for novel therapies that are effective, cheap and have negligible side effects becomes inevitable. Currently, standard drugs used in the management of pulmonary hypertension such as bosentan and sildenafil are expensive or not readily available in some parts of Nigeria. In addition, there are numerous reports of disappointing results with the use of these drugs in some clinical studies.[104,105] A multi-centre randomized placebo-controlled trial in adults and children with SCD-associated pulmonary hypertension using sildenafil was abruptly stopped due to an increase in incidence of vaso-occlusive crisis in patients on the sildenafil arm.[105] The favourable literature around $_{L}$-arginine as a possible panacea in the management of the pulmonary hypertension associated with SCD needs to be explored further.

1.5.2.3 Priapism

Priapism, a condition resulting from blood trapping in corpora cavernosa and affecting about a-third of children and adolescent with SCD, is thought to result from low NO bioavailability.[106] The existing treatment protocol is often unrewarding and search is ongoing for a better

treatment option. Although it is not conventional to offer arginine as therapy to patients with this condition, successful resolution of priapism was observed in an adolescent who received 100 mg/kg of parenteral arginine hydrochloride after 20 minutes of arginine infusion.[106] This observation provides a basis for further studies on the role of arginine in the management of priapism.

1.5.2.4 Acute chest syndrome

Nitric oxide deficiency plays a major role in the pathogenesis of ACS. This observation has drawn attention to the possibility of treating the condition with inhaled NO (iNO). Sullivan *et al*[107] reported the case of a young adolescent with ACS that was successfully treated with iNO. In response to this finding, a phase 2 clinical trial (Clinical Trial.gov Identifier: NCT00029731) on arginine treatment of ACS (aimed at generating NO through oral arginine supplementation) was conducted but the result of the study has not been published.

1.5.2.5 Vaso-occlusive painful crisis

Studies on arginine and pain control are rare. Following the discovery of low plasma arginine in children with VOC, a randomized placebo-controlled study on arginine in VOC was conducted which demonstrated a 54% reduction in opioid use and a 50% reduction in pain scores with arginine use compared with the control groups.[27] In addition, the arginine arm showed a trend towards shorter hospital stay.

The multiple effects of arginine supplementation demonstrated by the various reports corroborate the idea that NO pathway plays a central role in the vasculopathy of SCD. However, some of the reported effects could not be explained by NO alone, thus suggesting that the effects of arginine therapy transcend NO synthesis.

The summation of results of arginine therapy in SCD suggests that arginine may represent the long awaited novel therapy with multiple benefits. It is however disheartening that virtually all the studies on arginine supplementation in individuals with SCD were from developed countries. It is desirable that researchers in Africa, where 75% of global SCD patients reside, further explore the potential benefits of $_{L}$-arginine in the control of complications of SCD.

1.6 Rationale for the study

Sickle cell anaemia is a common chronic debilitating disease and Nigeria has been shown to have the largest number of children with the condition. Sickle cell anaemia is one of the highest non-infectious causes of mortality in Nigeria, and cardiopulmonary complications are the leading causes of death.[28-31,33]

There is paucity of data on the influence of sickle cell crises on cardiovascular parameters in children with SCA in Africa. Some cardiac changes that occur during sickle cell crises may contribute to the sudden death phenomenon in children with SCA. Notable cardiac complications include myocardial ischaemia, diastolic dysfunction, elevated pulmonary pressures and arrhythmias.

Myocardial ischaemia was thought to be rare in children with SCA even during crises but more recent reports suggest that the presumed rarity of MI in VOC and ACS may be due to missed diagnosis and under-reporting.[57-59]

Pain which is the clinical hallmark of VOC, is debilitating for patients and is associated with negative effects on health care resources, high hospital admission rates, and a high economic burden and loss of school hours. There is no effective treatment that targets the pathophysiologic mechanism of VOC. Management consists mainly of hydration of the patient and symptomatic relief with the administration of opioid analgesics which remains unsatisfactory due to the fear of opioid dependency which is compounded by inaccurate pain intensity assessments.

Severe painful crisis is a major cause of morbidity[19,20] and an important contributor to mortality in adults and children with SCA. Acute painful crisis is a trigger for the acute chest syndrome.[30,32,33] Acute chest syndrome is one of the leading causes of morbidity in SCA and the most common cause of mortality in these patients.[28-31] Both VOC and ACS cause acute increases in pulmonary pressures which can be measured indirectly using the echocardiographically derived tricuspid regurgitation velocity (TRV).[62,63] Even mild acute increases in pulmonary pressures could be devastating and may result in cardiovascular collapse and sudden death. Studies in adults with SCA have shown that TRV $\geq$ 2.5 m/s increases the risk of death by 10-fold and is a major independent risk factor for sudden death.[51]

Both VOC and ACS have been shown to have strong correlations with serum arginine levels and global arginine bioavailability and may require supplemental arginine to improve outcome.[87,90] Mean serum arginine concentration could drop from a mean of 53.6±4.6 µmol/l in steady state to a mean of 34.5±3.3 µmol/l in children hospitalized for VOC.[90] Arginine therapy has been reported to decrease pain scores and has also been shown to have a narcotic-sparing effect in children with VOC.[27] In addition, exogenous administration of arginine has been shown to improve NO production and hence an increased NO bioavailability necessary for vascular function, free radical scavenging, pulmonary vasodilatation, improved tissue perfusion and therefore limit many complications. Although these effects could be achieved with administration of inhaled nitric oxide (iNO), it is expensive, cumbersome to administer and not readily available.

$_{L}$-Arginine, which is the nitrogen donor for endogenous synthesis of nitric oxide, is less expensive and easy to administer. The multiple effects of $_{L}$-arginine in VOC and ACS make it a potentially indispensable agent in the management of SCD children. In addition, arginine supplementation enhances lymphocytic blastogenesis in SCD patients, a property that may improve immune functions and enhance infection control. $_{L}$-arginine is safe, well tolerated, can be obtained over-the-counter as a food supplement and does not require stringent control like iNO and other agents required in the management of SCA patients.[27]

Studies on arginine supplementation in SCA children have not yet been reported in Africa, despite the fact that the disease is prevalent and that it has a role in management of complications of SCA.

To date, there are no reports of studies evaluating the direct cardiovascular benefits of supplemental oral $_{L}$-arginine in children with VOC and ACS.

The present study is therefore aimed at characterizing the cardiac changes that occur during sickle cell crisis with particular interest in ventricular function, myocardial wall stress, myocardial ischaemia and the tricuspid regurgitant velocity. An additional focus will be to determine if oral arginine supplementation has a role in altering the cardiac changes as well as improving pain control in SCD subjects with VOC and ACS.

If the use of arginine is shown to be beneficial in the management of children with SCA, it could be added to existing national protocols for the management of the disease.

1.7 RESEARCH HYPOTHESIS

1. Sickle cell crisis creates an additional burden on the already stressed myocardium in children with sickle cell anaemia and it has the potential to alter ventricular function and pulmonary pressure.

2. Arginine supplementation improves pain control and cardiac sequelae of sickle cell crises.

1.8 Objective of the study

To study the effect of sickle cell crisis on cardiovascular parameters and the impact of arginine supplementation at the dose of 100 mg/kg/dose 8 hourly for 5 days on the pain and cardiac sequelae of severe vaso-occlusive crisis and acute chest syndrome in children, aged 5-17 years, with sickle cell anaemia.

1.8.1 Specific Objectives

1. To determine the influence of sickle cell crisis on the prevalence of myocardial ischaemia, cardiac dysfunction and elevated mean pulmonary artery in children with sickle cell anaemia.

2. To evaluate the impact of arginine supplementation on cardiac changes in vaso-occlusive crisis and acute chest syndrome.

3. To determine the role of arginine as adjuvant therapy in the management of pain in sickle cell vaso-occlusive crisis.

CHAPTER TWO

2.0 METHODS AND MATERIALS

2.1 Study location

This study was carried out in the Federal Capital Territory (FCT) of Nigeria, Abuja. The FCT is located at the centre of Nigeria and consist of 6 area councils, Figure 2.1, each having at least one government owned health facility that provides either secondary or tertiary health care.



Figure 2.1: Map of Nigeria showing the location of Abuja (figure above) and the 6 area councils (figure below). The two study sites are located in Gwagwalada and Abuja Municipal Area Councils

There is however some inequality in the distribution of health facilities. The largest concentration of health facilities is in the Abuja Municipal Area Council (AMAC) that has 6 secondary level health facilities and one tertiary health facility (National Hospital) as a result of high concentration of settlements in the area. Gwagwalada Area Council has one tertiary health institution (University of Abuja Teaching Hospital, UATH) while the other area councils have one secondary facility each.

Paediatric sickle cell clinics are held weekly in the National Hospital and UATH, and a total of 800 SCD patients are on the clinic registers. Patients attending SCD clinics are confirmed sickle cell disease patients, the majority (about 95%) of which are SCA patients. Children with mild VOC are treated in the clinic while those with severe presentations are hospitalised in the emergency unit in the respective hospitals. Those in a steady state are monitored in the clinic every 2-3 months. All the SCA patients are routinely offered folic acid (5 mg/day) and proguanil tablet as well as oral penicillin V to those who are ≤ 60 months old.

2.1.1 Institutional standard of care for patients with severe VOC

The pediatric units in the sites of study utilized a standard protocol for managing severe pain in patients with vaso-occlusive crisis, which included parenteral opioid at presentation, alternating with non-steroidal anti-inflammatory drugs (ketorolac, diclofenac) for all patients, regardless of study participation. In the University of Abuja Teaching Hospital (UATH), the commonly used opioid analgesic for severe pains is parenteral pentazocine. This is used in a staggered dose format with intramuscular diclofenac/ketorolac. Parenteral morphine is not readily available.

At the National Hospital, parenteral pentazocine is the commonly used opioid in a treatment regimen that includes alternating with parenteral/suppository diclofenac sodium. Liquid oral morphine is often introduced very early in the treatment to replace pentazocine. However, oral morphine is rarely used in the UATH, parenteral morphine is not readily available, and patients seldom receive adjuvants such as diazepam.

When the pain is subsiding, patients are taken off opioids and maintained on different combinations of diclofenac (suppository or tablet), Ibuprofen tablet, and acetaminophen tablet alternating with dihydrocodeine.

Hydration of patients is universal, unless otherwise indicated.

Malaria is holoendemic in North-central Nigeria, hence patients with severe crisis are empirically treated with antimalarial agents. Those patients showing features of superimposed infection(s) are given empirical antibiotics until laboratory results are available to allow the opportunity to change antibiotics according to the sensitivity of the organism.

2.2 Study design

The study is a prospective, multifaceted study involving children with sickle cell crisis and proceeded in stages as follows:

2.2.1 To determine the influence of sickle cell crisis on cardiovascular parameters of children with SCA.

This is a comparative case-controlled study involving age- and sex-matched sickle cell anaemia children with any form of crisis (cases) and sickle cell anaemia children in steady state (controls), from October 2016 to February 2019. Children in a steady state (HbSS patients that have not had any acute illness in the preceding 6-8 weeks) were obtained from the weekly held sickle cell clinic. The cases with sickle cell crisis were recruited from the Emergency Paediatric Unit.

Enrolment Criteria:

Any child, 5-17 years old, with HbSS genotype who presented with complaints of pains characteristic of VOC or features of any other forms of sickle cell crisis such as difficulty with breathing (ACS), worsening pallor/jaundice (hyperhaemolytic crisis) or stroke (acute cerebral syndrome) in whom parental consent and assent (from the child) was available were recruited.

Exclusion criteria:

Children with concomitant acquired heart diseases (such as rheumatic heart disease, Kawasaki disease, cardiac rhythm abnormalities) and congenital heart defects (such as ventricular septal defects, patent ductus arteriosus, coarctation of the aorta) were excluded as well as those with severe acute malnutrition, serum bicarbonate < 16.0 mmol/L, serum potassium < 3.1 mmol/L or > 5.5 mmol/L and endocrine abnormalities such as hypo/hyperthyroidism.

Sample size calculation

Sample size calculation was based on the prevalence of myocardial ischaemia in children with VOC (89.5%) and those in steady state (72.5%) based on the myocardial ischaemia score developed by Bode-Thomas *et al*.[57] Using a power of 80% and 5% level of significance, a sample size of 84 patients, for the cases and for the control was needed (total of 168). An additional 8 patients in steady states requested to participate in the study for the purpose of knowing their cardiac condition. Thus, a total sample size of 176 patients was obtained.

Study Outcome Variables

(i) **Primary outcome**:

Proportion of children with myocardial ischaemia as determined by electrocardiography and cardiac biomarkers (cardiac troponin I).

(ii) **Secondary outcomes**:

(a) Prevalence of systolic and diastolic cardiac dysfunction using M-mode and Doppler parameters such as fractional shortening, ejection fraction, Tei index, mitral and tricuspid annular planar systolic excursions, and mitral and tricuspid inflow velocities.

(b) Proportion of children with tricuspid regurgitation velocity >2.5 m/s and Doppler estimated mean pulmonary artery pressure >25 mmHg.

Study Procedure: Children that fulfilled the inclusion criteria were recruited consecutively until the sample size was obtained. The patients were subjected to pulse oximetry at presentation, and also had standard 12-lead surface electrocardiography and transthoracic echocardiography. Blood samples were obtained from the antecubital fossa and placed into lithium heparin bottles and analysed for haemoglobin concentration and cardiac troponin I.

2.2.2 To evaluate the impact of arginine supplementation on cardiac changes in acute vaso-occlusive crisis and acute chest syndrome:

Children with severe VOC (pain score of 7 and more) and ACS admitted into the emergency units of National Hospital and UATH were recruited.

Trial design and participants

The trial was designed as a prospective, double-blind, randomised, placebo-controlled, phase II study (RCT) of oral arginine supplementation involving children with SCA having VOC with or without acute chest syndrome (ACS), from February 2017- February, 2019.

Inclusion criteria:

(i) Diagnosis of SCA in a child aged 5-17 years.

(ii) Sickle cell painful crisis with Numerical Pain Rating Score (NRS) of ≥7 on a 0-10 point scale (NRS was validated for use in Nigerian children by Eyelade *et al* in 2009).[108]

(iii) Willingness to participate in the study (informed written consent and assent)

Exclusion criteria:

(i) Patients with acquired and congenital heart diseases such as rheumatic heart disease, dilated cardiomyopathy, septal defects and cardiac outflow obstruction.

(ii) Patients that required immediate red cell transfusion.

(iii) Patients with a known or suspected allergy to arginine.

(iv) Sickle cell anaemia admitted for reasons other than VOC and ACS.

(v) Inability to provide written and dated consent/assent.

Sample size calculation was based on the data published by Morris *et al*[35] on arginine therapy for pulmonary hypertension in SCD. Using pulmonary artery systolic pressure mean of 63.9 mmHg,[35] power of 80%, a significance level of 5%, a sample size of 33 patients each in the arginine and placebo groups (total of 66 patients) was adequate to detect a mean change of 10 mmHg in Doppler estimated pulmonary artery systolic pressures with the assumption that the response of pulmonary artery systolic pressure would be similar to that observed by Morris *et al.*[35]

Study Procedures

Children admitted into the Emergency Paediatric Unit with the diagnosis of sickle cell crisis were evaluated clinically to determine the type of crisis. Those with painful episodes consistent with acute vaso-occlusive crisis and acute chest syndrome (defined by chest pain and breathing difficulty, and/or new infiltrates on chest radiography) were recruited, and underwent anthropometry, pulse oximetry, blood pressure measurement, surface electrocardiography and transthoracic echocardiography which were done by the researcher as soon as the patients became calm. Blood samples for cardiac biomarkers (cardiac troponin I and NT-pro BNP) and arginine bioavailability (plasma arginine, ornithine, citrulline) were obtained at the time that intravenous access for medications was achieved.

Randomisation and masking

The patients were randomized into an oral $_{L}$-arginine arm and a placebo (sucrose) arm of treatment by a block of 4 randomisation design. The arginine and placebo were provided in visually identical packets with a pre-determined dose. The masking and randomisation process was done by the pharmacist in the Drug Compounding Unit trained in the permuted block randomization technique to ensure that the patients, the dispensing pharmacist/or nurse and researcher were blinded to the product administered.

Enrolment and dispensing

Baseline parameters such as the pulse/heart rate, blood pressure, oxygen saturation, electrocardiography and echocardiography were documented. Blood was taken for the panel of plasma amino acids which were of interest. A pharmaceutical grade $_{L}$-arginine-hydrochloride powder (1119-34-2, Cat/Case No. A4599, Ajinomoto Co. Inc., USA), or the placebo (sucrose) was administered orally at 100 mg/kg/dose, 8 hourly, for maximum of 5 days or until patient discharge, whichever came first. The administration of the arginine and placebo was done by a blinded pharmacist and/or a nurse. The study drug was dissolved in red grape juice and ingested immediately. Administration of the products did not interfere with the unit protocol for managing patients with VOC and ACS.

Monitoring

The patients were monitored daily and assessed for presence of adverse drug reactions as well as oxygen saturation (pulse oximetry) and blood pressure pattern. On the 6^{th} day of hospitalisation, the patients had a repeat of pulse oximetry, blood pressure measurement, electrocardiography, echocardiography and plasma analysis for cardiac troponin I, NT-pro BNP, arginine, ornithine and citrulline levels.

Trial Oversight

Approval from all related regulatory bodies, including the National Agency for Food and Drug Administration and Control, were obtained before commencement of the study (see appendix 1).

Outcome variables

Primary outcome:

Percent change in echocardiographically-derived mean pulmonary artery pressure.

Secondary outcomes:

(a) Percent change in levels of cardiac biomarkers (Troponin I, NT-pro BNP).

(b) Changes in ventricular wall stress.

(c) Relationship between elevated tricuspid regurgitation velocity (TRV >2.5 m/s) and arginine-ornithine ratio (index of arginine bioavailability).

(d) Changes in echocardiographic indices of ventricular functions such as fractional shortening, ejection fraction, Tei index, mitral and tricuspid annular planar systolic excursions, and mitral and tricuspid inflow velocities.

2.2.3 To determine the role of arginine as adjuvant therapy in the management of pain in sickle cell vaso-occlusive crisis.

Children with severe VOC admitted into the emergency units of National Hospital and UATH were recruited, some of whom participated in the study on the effect of arginine supplementation on the cardiovascular changes in sickle cell crisis.

Trial design and participants

A prospective, blinded, randomized, placebo-controlled, phase II trial was designed to detect a 50% reduction in opioid requirement per episode of severe VOC, from February 2017 to February 2019.

Inclusion criteria:

(i) Diagnosis of sickle cell anaemia in a child aged 5-17 years.

(ii) Pain location in one or more sites typical of VOC.

(iii) Numerical Pain Rating Score of $\geq$7 on a 0-10 point scale (validated for use in Nigerian children by Eyelade *et al*, 2009).[108]

(iv) Willingness to participate in the study (informed written consent and assent).

Exclusion criteria:

(i) Presentation with non-VOC pain such as headache, pain due to trauma, fracture or deep venous thrombosis.

(ii) Clinical findings suggestive of osteomyelitis.

(iii) Patients with neurologic impairments such as impaired vision, hearing, or motor function deficit.

(iv) Patients with known/suspected allergy to arginine.

(v) Sickle cell anaemia admitted for reasons other than VOC.

(vi) Failure to provide written and dated consent/assent.

Sample Size Determination

Sample size was determined based on data published by Morris *et al*[27] on arginine therapy in children with VOC episodes. Using a standard deviation of 3.0, significance level of 5%, a power of 80%, 34 patients each, total of 68 patients, was required in the arginine and placebo groups to detect a 50% reduction in the total dose of opioid needed for VOC pain resolution.

Randomization and masking

Randomization numbers were generated by Microsoft Office Excel. Using a block randomised design (block size of 4), patients were randomized in a 1:1 ratio of placebo to L-arginine as soon as they presented to the Emergency Department (ED) or within 6 hours of admission.

Enrolment and dispensing

The supplement (L-arginine-hydrochloride) and the placebo (sucrose) were provided in visually identical packets with a pre-determined dose, administered by a blinded pharmacist and/or a nurse. A pharmaceutical grade L-arginine powder (1119-34-2, Cat/Case No. A4599) purchased from Ajinomoto Co. Inc., USA, was administered every 8 hours (100 mg/kg/dose) for maximum of 5 days/15 doses or until the patient was discharge, whichever came first. Red grape juice was used to dissolve the study drug and the patient was made to consume the juice immediately under the supervision of the research pharmacist or nurse. The patients were followed up at 24 hours (day 1), and daily (days 2-5) after commencement of the study to capture adverse events, the daily worst and least pain score experienced and the pain intensity at the time of the evaluation (current pain).

Patients received institutional standard of care therapy. The cumulative dose of analgesics administered was computed daily and scored using the validated analgesic medication quantification scale (MQS)[25] while total opioid intake was computed using morphine-equivalent units/kilogram.

Trial oversight

The study was approved by the National Agency for Food and Drug Administration and Control (NAFDAC), Ref No: NAF/DER/CT/LAG/2017, by the Human Research Ethics Committees of the University of the Witwatersrand (M 160326), University of Abuja Teaching Hospital, Abuja (Ref No: FCT/UATH/HREC/PR/517) and the National Hospital (Ref No: NHA/EC/090/2016). The study was covered by an AIICO Insurance Policy, (No: 310/00001/17/TQ/HO) and it was registered at the Pan African Clinical Trial Registry (PACTR201611001864290).

An independent validation of the final database was performed by the Emory University, Atlanta, Georgia, Department of Pediatrics Biostatistical Core.

Safety monitoring

An independent Data Safety and Monitoring Board (DSMB), consisting of Dr. Simon Kene Terfa (a Community Health Physician with expertise in medical statistics and who works in the Centre for Disease Control, Abuja), Professor Augustine Odili (a Cardiologist from Department of Internal Medicine, UATH) and Dr. Uduak Offiong (a Paediatrician, from Department of Paediatirics, UATH), reviewed the study for data safety. The occurrence of two Serious Adverse Events related to the study medication was the main research stopping criterion and the DSMB held the research stopping right. Adverse events were defined according to the Medical Dictionary for Regulatory Activities version 12.0. The data was first analysed with the statistician blinded to the code of the study products before unblinding of the study.

End points

1. The **primary efficacy end point** was the change in MQS, a sensitive measure to quantify analgesic use in patients with SCA.[25]

2. **Secondary outcomes** included differences in percentage of analgesic use, change in mean pain scores, time-to-crisis-resolution (defined as time from study drug randomization to pain score <4), and the total length of hospital stay across the study arms. Crisis resolution was defined as a worst pain score <4 (using Numerical Pain Rating Scale). Time-to-crisis-resolution in patients with missing end-point data due to early discharge were censored at the actual time of discharge. Death before meeting the criteria for crisis resolution was censored at the latest time-to-crisis-resolution for participants who were discharged alive.

2.3 Detailed laboratory procedures

2.3.1 Blood pressure measurement

A supine brachial blood pressure was obtained manually with an appropriate size cuff of the mercury sphygmomanometer (AccosonDekamet®, A.C. Cossor and Son Surgical Ltd, Accoson Works, Harlow, Essex) applied to the right arm. The blood pressure measurements were done

using the auscultatory method in line with the recommendation of the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents.[109] The sphygmomanometer's pressure markings that corresponded with the first and fifth Korotkoff sounds were recorded as the systolic and diastolic blood pressure, respectively. The average of three readings was recorded as the final blood pressure. The results were interpreted according to normative referenced values.[109] Systolic and diastolic blood pressures were defined as normal when the value fell below the 90th percentile for age, sex and height. Blood pressures between the 90th and 95th percentiles were considered to be elevated, while those greater than 95 percentiles and up to 99th +5 mmHg, and those greater than 99th + 5 mmHg were termed stage 1 and stage 2 hypertension, respectively.[109] However, in children aged 12 years and above, blood pressures greater than 120/80 mmHg were also considered elevated even if they are below the 90th percentile.[109]

2.3.2 Electrocardiographic evaluation[110]

The procedure of doing an ECG was explained to each parent/child, with a demonstration to reassure them of its safety and to calm the child. A conventional 12-lead resting ECG was then recorded with the child lying on a couch in supine position, according to standard procedure. A minimum of five cardiac cycles were recorded per ECG lead and where recordings were not clear, a recording was repeated. Where deflections were small or too large, standardization was doubled or halved respectively in order to identify the wave pattern. All ECG measurements were done manually by the investigator with the aid of a magnifying lens according to standard guidelines and values obtained were interpreted based on the normal paediatric ECG patterns for Nigerian children or Caucasian children where Nigerian values were not available.

The following measurements were taken from the ECG tracings: heart rate, the axis, P-wave duration and amplitude, P-wave dispersions, PR intervals, QRS duration and amplitude, QTc intervals and ST segment abnormalities.

- The heart rate was calculated using standard methods: the small box method for those with sinus rhythm and the 6-second method for those with irregular rhythm pattern.[102] In the small box method, the heart rate is equal to 1500/n; where 'n' represents the number of small boxes between the R-R interval. In the 6-second method, the heart rate is equal to the number of QRS complexes in a 6-second length of strip, multiplied by 10.

- The cardiac rhythm was defined as 'sinus rhythm' when a single P wave preceded each QRS complex and the P axis was in the range of 0 to +90 degrees. Abnormal or non-sinus rhythm was defined by the presence of either abnormal number or shape of the P waves or abnormal axis.[110]
- PR interval was measured in lead II, from the beginning of the P wave to the beginning of the QRS complex, irrespective of whether the QRS complex began with a Q or R wave. PR intervals longer than the upper limit of normal for age as obtained in normal ECG values published by Kolawole *et al*/Davignon *et al,* were considered prolonged (i.e. first degree heart block).[111,112]
- QRS complex duration was measured in V_1 from the beginning of Q wave to the end of S wave along the isoelectric line on the ECG tracing. A QRS durations longer than the upper limit for age as contained in the normal ECG values published by Kolawole *et al*[112] and Davignon *et al,*[111] were considered as right bundle branch block (RBBB). Other criteria for RBBB include right axis deviation (RAD), at least for the terminal portion of the QRS complex; terminal slurring of the QRS complex (wide and slurred S waves in leads I, V_5 and V_6 or terminal, slurred R' in aVR and the right precordial leads).
- QRS amplitude was measured in the limb leads and some precordial leads. R waves taller than the 98th percentile for age in the right precordial leads and S waves deeper than the 98th percentile in the left precordial leads was taken as right ventricular hypertrophy (RVH). Additional criteria for RVH were the presence of RAD for age or abnormal R/S ratio in favour of the RV (in the absence of bundle branch block): R/S ratio in V_1 and V_2 greater than the upper limit of normal for age.[110]
- ST-segment was taken as the interval between the J point (or end of QRS complex) and the beginning of the T wave (defined as the point of maximum abrupt deflection after the ST-segment). ST segment elevation or depression was taken as a shift >1 mm in the limb leads or a shift of >2 mm in the precordial leads, from the isoelectric line.[110,111]
- QT intervals were measured in lead II or V_5 (depending on where there was a Q wave and a clearly defined T wave) from the beginning of the QRS complex to the end of the T wave and it was corrected for heart rate using Bazett's formula: $QTc=QT/\sqrt{(RR)}$. The QTc interval was defined as prolonged if it exceeded 0.44 seconds.[111]
- Myocardial ischaemia score was then computed following the recommendation of Bode-Thomas *et al*[57] as indicated below:

Criteria for derivation of myocardial ischaemia (MI) scores from standard 12-lead electrocardiograms.

Parameter	Score
Any non-sinus rhythm	1
Conduction disturbances*	1
QTc>0.440 seconds	1
QRS-T angle >90 degrees	1
ST elevation or depression >1 mm in 1 or 2 leads	1
ST elevation or depression >1 mm in 3 or 4 leads	2
ST elevation or depression >1 mm in ≥5 leads	3
Flat, diphasic or inverted T-waves in 1 or 2 leads	1
Flat, diphasic or inverted T-waves in 3 or 4 leads	2
Flat, diphasic or inverted T-waves in ≥5 leads	3

*PR interval >0.16s, QRS duration ≥0.1s, sinus pauses with or without escape beats or rhythms.

The maximum score obtainable by any patient was 10 and the minimum was zero. Significant myocardial ischaemia, corresponds to a score of >3. MI was considered to be present if the Q-wave was abnormal in any lead (duration >0.04 seconds with or without notching), if the ST-segment was elevated beyond 2 mm or if the corrected QT interval (QTc) exceeded 0.440 seconds with accompanying Q-wave abnormalities.

2.3.3 Echocardiographic evaluation technique[72,113,114]

Standard two dimensional, M-mode, and Doppler echocardiograms was performed by the researcher in the supine and left lateral decubitus positions and measurements done as recommended by the American Society of Echocardiography using a GE Vivid-e echocardiography machine with high resolution 5-7.5 MHz sequential transducer and calculation package. The patients were calmed but not sedated. Standard parasternal, apical, subcostal, and suprasternal views were used. Chamber dimensions, valvular morphology, functions and flow were assessed while parameters of systolic and diastolic functions were documented. Calculations not done by the GE Vivid-e machine were performed manually.

Chamber size quantification: Simultaneously recorded ECG was used as a reference to accurately determine end systole and end diastole. Linear measurements of internal left

ventricular (LV) diameter, septal and inferior-lateral wall thickness at end diastole [(LVIDd), (IVSd), (LVPWd)] and end systole [(LVIDs), (IVSs), (LVPWs)] respectively, were obtained using M-mode measurements from the parasternal short axis view just below the tips of the mitral valve leaflets and were indexed to the body surface area. LV dilatation was defined as LV end-diastolic dimension (LVIDd) z-score >2 using Kampmann *et al*'s criteria.[115]

Valvular morphology and flow: the atrioventricular (AV) valves were measured from the apical 4-chamber view using the hinge point of the valve to the septum or lateral walls as the landmark. Aortic valve annulus was measured in the parasternal long axis left ventricular outflow tract (LVOT) view while the pulmonary valve was measured from the parasternal long axis right ventricular outflow tract (RVOT) view. Transvalvular flow gradient was assessed in the same views as the annulus measurement. Tricuspid regurgitation was assessed in the parasternal right ventricular inflow, parasternal short-axis and apical four-chamber views. A minimum of three sequential complexes were recorded. Stroke volume (SV) was calculated by multiplying LVOT time velocity integral, determined by pulse wave Doppler (PWD), by the LVOT cross-sectional area while the cardiac output (CO) was gotten by multiplying the SV by heart rate. This method is based on the assumption that the LVOT is circular, while in real fact it is ellipsoidal, thus, the method is prone to underestimation of the true SV. Measurement of the zoomed LVOT dimension at the hinges of the aortic valve and the mean LVOT VTI of 3-5 most distinct spectral envelopes of the flow limits the error of underestimation of the SV. As an alternative, stroke volume may be calculated using the Teichholz formula.[116]

Continuous-wave Doppler (CWDoppler) sampling of the peak regurgitant jet velocity was used to estimate the right-ventricular systolic pressure (RVSP) with the use of the Modified Bernoulli equation. Pulmonary artery systolic pressure (PASP) was calculated from the tricuspid regurgitation velocity (TRV) and the estimated right atrial pressure (RAP) using the Modified Bernoulli equation:

Pulmonary Artery Systolic Pressure = **4 (TRV)** ^{2} **+ RAP**.

The mean RAP was estimated according to the degree of collapse of the inferior vena cava (IVC) with inspiration: 5 mmHg for a collapse of greater than 50%, 10 mmHg for a collapse of 25-50% and 15 mmHg for a collapse of less than 25%.

Estimated pulmonary artery end-diastolic pressures (PADP) was obtained by applying the Modified Bernoulli equation to the pulmonary regurgitation (PR) end-diastolic velocity Doppler signal and assuming the estimated end-diastolic RV pressure is equal to the estimated RA pressure. No PR measurement was done without parallel alignment of Doppler signal. Patients with acquired heart diseases like constrictive pericarditis were excluded to avoid underestimation of PR signal.

Mean pulmonary artery pressure (MPAP) was calculated from the classic rule of thumb formula:

Mean pulmonary artery pressure = **2/3PADP + 1/3PASP**[66]

Estimation of PASP from TRV is dependent on the angle of interrogation and the presence of adequate Doppler envelope, consequently the error of underestimation is possible without these requirements being fulfilled. Furthermore, the RVOT must not be obstructed, hence patients with pulmonary stenosis were excluded. The TRV was obtained from multiple views including the RV-focused apical four chamber view to get the best Doppler angle and the highest velocity, and a frame rate ≥20 Hz with colour Doppler was used.[66] The option of using intravenous agitated saline to improve the TR signal was not used in any patient.

In order to avoid overestimation of RAP, only the IVC collapsibility index was used. Other alternative formulas[66] that could be used in patients that failed the classic rule of thumb criteria included:

MPAP = 0.61 (PASP) + 2 mmHg (Chemla formula).

MPAP = 4 (PRpeak velocity)^2 + RAP.

MPAP = 90 – (0.62 (AT_{RVOT}), where 'AT_{RVOT}' is the RVOT acceleration time.

Pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) were obtained using the equation:

Pulmonary Vascular Resistance (Woods Unit) **= [*V*(TR) max⁄$VTI_{(RVOT)}$ × 10] + 0.16**[117]

Where V(TR)max = maximum tricuspid regurgitation velocity, $VTI_{(RVOT)}$ = velocity time integral of right ventricular outflow tract.

Although, PVR determined by echocardiography correlates well with values obtained from cardiac catheterisation, anatomic modification of right heart structures may make accurate measurements of the $VTI_{(RVOT)}$ difficult.[118] In patients with severe PVR, this method may also be inaccurate. Therefore, the interpretation of PVR measurements was done with caution. Cardiac catheterization remains the gold standard in determining PVR.

Systemic Vascular Resistance (Woods Unit) **= Mean Arterial Pressure ÷ Cardiac Index**[119]

Left Ventricular Systolic Function- parameters of systolic function such as Fractional shortening (%FS), Ejection fraction (EF) and velocity of circumferential fibre shortening (VcFc) were obtained from the echo machine reading or calculated manually. Systolic function was considered abnormal if the fractional shortening was less than 28% or the ejection fraction was less than 55%.[72,114] Harmonic imaging technique was used to improve endocardial border detection and to lessen foreshortening. Intravenous contrast administration was not used in any patient.

Right Ventricular Systolic function- percent fractional area change (%FAC) was obtained from an apical 4-chambered view. %FAC < 33% was considered abnormal.

Meridional wall stress (WS), defined as force per unit area acting at the equatorial plane of the ventricle in the direction of the apex to base axis was determined from the equation: [71,72,114]

Left ventricular wall stress, circumferential, (gm/cm^2)

$$= \frac{1.35Pr[1-(2r^2/L^2)]}{LVPWs}$$

Where P = systolic cuff pressure, r = LVIDs/2, L = left ventricular maximum major axis in apical four chamber or two chamber view.

Left ventricular wall stress, meridional, (gm/cm^2)

$$= \frac{0.334(P \times LVIDs)}{LVPWs[1+(LVPWs/LVIDs)]}$$

Where P = systolic cuff pressure, LVIDs = left ventricular internal diameter in end-systole, LVPWs = left ventricular posterior wall thickness in end-systole.

Measure of global LV and RV functions: The Myocardial Performance Index (MPI) was derived by pulsed-wave Doppler. The time intervals from valve closure to valve opening for

the mitral and tricuspid valves were used to determine the total systolic duration (TSD) for the LV and RV, respectively. The LV and RV ejection times (ET) were measured from the onset to end of LV and pulmonary outflow, respectively. MPI was calculated as TSD-ET/ET for each ventricle. Normal MPI value for LV is 0.35±0.03 and for the RV is 0.28±0.04.[72,114] The MPI however, does have disadvantages. It is affected by changes in loading conditions and has paradoxical change with high filling pressure or severe semilunar valve regurgitation. Hence it is not suitable for the evaluation of LV function in patients with atrioventricular block, atrial fibrillation or flutter, severe mitral and aortic valve disease, and severe pericardial effusion.[120] Therefore, patients with these conditions were excluded. In addition, MPI does not readily discriminate abnormalities of systolic function from those of diastolic function.

Pulsed Tissue Doppler velocity of the lateral mitral and tricuspid annulus was used to determine other parameters of systolic and diastolic functions while M-mode measurement of mitral/tricuspid annular plane systolic excursion (MAPSE/TAPSE)[72,121,122] was used to assess LV/RV systolic function, respectively.

Measurement of diastolic function: Pulsed Doppler mitral and tricuspid inflow velocities (early E and late A waves) were measured at the tip of the mitral and tricuspid valves, respectively and the early-to-late diastolic flow (E/A) ratios were computed. The pulmonary venous flow velocities (S-wave and D-wave) were measured at the right upper pulmonary vein and the systolic-to-diastolic peak velocity (S/D) ratio was calculated. Lateral peak early diastolic tissue velocity (E') was assessed at the mitral annulus and the mitral E-to-E' (E/E') ratio determined. Using the Nagueh formula, the pulmonary capillary wedge pressure (PCWP) was calculated as: **PCWP =1.9 + 1.24 (E/E').**

It is worth noting that TDI velocities assess mainly longitudinal ventricular function, thus may not correlate well with measures of ventricular functions assessed by other methods. Combining mitral inflow Doppler with pulmonary venous Doppler provides a more comprehensive assessment of LA and LV filling pressures.

Echocardiography imaging, storage and analysis: Echocardiography was performed when the patient was calmed. Images were acquired during quiet respiration (or at end-expiration apnoea in line with standard recommendations),[123,124] analysed online, stored and re-analysed offline. The two values obtained were compared to determine intra-observer variability.

2.3.4 Serum biochemical analysis: About 5 ml of venous blood was collected into a lithium heparin bottle from the ante-cubital vein using sterile, disposable syringes. The blood samples were centrifuged at 3500 rpm for 15 minutes by the researcher or trained assistant. The plasma was decanted into a test tube and kept frozen at -80^{o}C until analysed. The plasma samples were transported in dry ice to South Africa. Analysis for plasma arginine, ornithine and citruline was performed at the Centre for Human Metabolomics, Department of Biochemistry, North-West University, Potchefstroom, South Africa using a High Performance Liquid Chromatography-Mass Spectrometry (LC-MS-MS) system and the amino acids detected via multiple-reaction-monitor.

Specimen for plasma NT-pro BNP and cardiac troponin I was also stored at -80^{o}C until analysed with a fully automated electrochemiluminescence immunoassay method using Maglumi® 800 analyzer (Snibe Diagnostics, Shenzen Mindray Biomedical, China) with the assistance of a laboratory scientist, blinded to the clinical information of the patients, at the Chemical Pathology Laboratory of UATH, Abuja.

2.4 Quality Assurance

Instruments for anthropometry were calibrated against the standards used before. The research assistants were trained and monitored closely to minimise inter-observer and intra-observer variations. Each patient was handled by the same research assistant. Intra-observer variances were examined periodically and controlled. Electrocardiography and echocardiography were performed by the researcher, who has had a 6-month refresher course in paediatric echocardiography at the Paediatric Cardiology Unit of Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg using the guidelines and standards for performance of paediatric echocardiogram as proposed by the Task force of the Pediatric Council of the American Society of Echocardiography,[113] before the commencement of the study. The plasma samples were kept in a high performance ultra-low freezer and the biochemical analysis was performed in an accredited centre by certified scientists and professionals.

2.5 Statistical Analysis

2.5.1 Adherence and protocol deviations

2.5.1.1 P*rotocol deviations summarised*

The sample size for the study was calculated manually using formulas but when the STATA 14 (StatCorp, LP, College Station, TX, USA) software was used, it was found that fewer numbers of patients were needed to detect significant change in the desired outcome variables. For objective 1, the required sample size based on STATA 14 software was 168 patients rather than 210 obtained by manual calculation. Similarly, for objective 2, a sample size of 66 patients was sufficient to detect the effects of arginine supplementation on the mean Doppler estimated pulmonary artery pressure as against 100 patients earlier calculated, while 68 patients were found sufficient to detect significant reduction in total analgesic medication required in the management of a painful vaso-occlusive episode following arginine supplementation. Therefore, the sample size was adjusted to reflect the numbers obtained with STATA 14 software.

2.5.2 Missing data

The study protocol was designed to minimize the effect of missing data. However, clinical practice has shown that obtaining data from children can be tedious and the completeness of the data unpredictable. Therefore, missing data for variables, due to challenges arising from obtaining measurements during painful crisis despite optimal analgesic, was anticipated; for example, patients may become irritable and uncooperative making complete capture of desired echocardiographic data unlikely. Any patient whose data was completely missing was excluded from analysis while those who had a few missing data points were only exempted from analysis at the point of missing data.

2.5.3 Assumed confounding covariates

The variables measured in the study may be related, and may affect each other and the outcome. Beside the measured variables, other variables dictated by environmental, patient's genetics and psychological factors may affect the final outcome, and were considered unmeasured variables.

- Arginine levels were assumed to be confounded by:
    - ❖ Variation in the level of arginine in patient's diet: unmeasured
    - ❖ Differences in endogenous arginine production: unmeasured
    - ❖ Exogenous arginine supply: measured
- Changes in pain levels were assumed to be cofound by:
    - ❖ Analgesic medication received: measured
    - ❖ Inherent patient's pain threshold: unmeasured
    - ❖ Severity of pain: measured
- Length of hospital stay was likely to be confounded by:
    - ❖ Comorbid conditions: unmeasured

It is acknowledged that there will be other confounding factors that were not captured. However, the variables captured were those that are regularly measured and are of interest in clinical practice.

2.5.4 Statistical analysis for cardiovascular changes in sickle cell crisis and impact of arginine supplementation on cardiovascular changes in sickle cell painful crisis and acute chest syndrome

The comparative case-controlled study of cardiovascular changes in sickle cell crisis and the phase 2 randomised controlled trial on the impact of arginine supplementation on the cardiac changes in vaso-occlusive pain crisis and acute chest syndrome were designed to have a power of 80%. Cardiac dimensions obtained from the patients were indexed to the body surface area and compared to values from published nomogram[121,122] and each observed value expressed as a standard deviation (SD) from the normal predicted value (Z-score).

2.5.4.1 *Definition of cardiac outcomes of interest*

Myocardial ischaemia was defined based on composite variables obtained from 12-lead standard surface electrocardiography and was considered present if the myocardial ischaemia score was >3.[57]

N-terminal prohormone brain-type natriuretic peptide (NT-pro BNP) was considered elevated when the plasma levels were 160 pg/ml and above[125] while troponin I levels of 0.2 ng/ml and above[113] were considered abnormal.

Other cardiac parameters of interest were defined as:

- Elevated pulmonary artery pressure: tricuspid regurgitation velocity >2.5 m/s, Doppler-derived pulmonary artery systolic pressure >30 mmHg, mean pulmonary artery pressure >25 mmHg.[126-128]
- Right ventricular dysfunction: right ventricular fractional area change <35%, right ventricular Tei index >0.40, tricuspid annular plane systolic excursion z-score (TAPSEZ) <-2SD.[72,122]
- Left ventricular systolic dysfunction: left ventricular ejection fraction <55%, left ventricular Tei Index >0.45,[129] mitral annular plane systolic excursion z-score (MAPSEZ) <-2SD.[72,121]
- Left ventricular diastolic dysfunction: mitral inflow E/A <0.8 or >2.5[113]

2.5.4.2 Test of Association and Reproducibility

Descriptive statistics were reported as mean±SD for continuous data and counts with percentages for categorical data. Mean values of heart rates, blood pressures and oxygen saturation during crisis and steady states were compared using Student's t-test. A paired t-test was used for pre- versus post supplement comparisons for variables with normal distribution. Normality was tested using the Shapiro-Wilk test and the Wilcoxon signed-rank test was used for comparing the plasma levels of troponin I and NT-proBNP among patients with elevated indices of pulmonary haemodynamics and other skewed continuous data.

The associations between myocardial ischaemia score and variables of the pulmonary and systemic circulations were tested using Pearson's correlation coefficients. The chi squared test of independence was used to test for differences between categorical variables. Data was analysed using STATA 14 (StatCorp, LP, College Station, TX, USA) and Minitab-17 (Minitab, LLC, State College, Pennsylvania). A p value of less than 0.05 was set as level of statistical significance.

Intra-observer reproducibility of echocardiographic measurements was tested using Bland and Altman agreement analysis.

2.5.5 Statistical analysis for data obtained on the role of arginine as adjuvant therapy

The trial was designed to have 80% power to detect a 50% difference in total analgesic medication use, based on data published by Morris *et al.*[27] Although total parenteral opioid usage was used to calculate the sample size, the medication quantification scale was chosen as a surrogate due to differences in practice between the United States and Nigeria; patients in the Nigerian study centres are commonly given only one or two doses of parenteral opioid at the time of presentation to the ED for severe VOC, and then rapidly switched over to oral medications. The switch over to oral medications is therefore not related to any change in patient condition. Data was analysed, with an "intention-to-treat," using STATA 14 (StatCorp, LP, College Station, TX, USA) and Minitab 17 (Minitab, LLC, State College, Pennsylvania) statistical software.

2.5.5.1 Test of Associations and Relationships

Normality of the continuous variables was assessed using the Shapiro-Wilk test. Differences between baseline continuous variables amongst the arginine and placebo groups were assessed using the Student's t-test or Mann-Whitney-U test, depending on normality, whereas the Pearson's Chi-squared test (or Fisher's exact, where the expected cells were less than 5) was used to compare categorical variables across the study groups. For repeated measurements, such as daily pain scores, the paired t-test was used for normally distributed continuous data and the Wilcoxon signed-rank test was used for skewed continuous data. The Pearson's correlation coefficient was used to assess the linear relationship between some continuous variables as well as the intensity of pain between the intervention and placebo arms. Association between variables was considered strong if the coefficient of correlation was at least 0.70, while those below 0.30 were considered weak and those between 0.30 and 0.60, moderate. Scatter plots with line of best fit were drawn to test the relationship between some continuous variables and medication quantification scale scores between the placebo and the intervention arms. The time-to-crisis-resolution as well as time-to-discharge between the two groups was compared by using Kaplan-Meier plots and log-rank tests. The total daily and cumulative analgesic intake as well as the daily decline in pain scores between the two study groups were analysed using a linear mixed model. A $p<0.05$ reflected statistical significance.

2.5.6 Multiplicity

In the same cohort, two primary outcomes were tested for significance thereby creating a multiplicity issue. An adjustment for multiplicity was applied based on the total numbers of different primary outcomes tested. A Bonferroni adjustment of the p-value was considered too conservative for the study. Therefore, the Jakobsen *et al*'s approach[130] to multiplicity adjustment threshold was followed. In this approach, "dividing the pre-specified p-value threshold with the value halfway between 1 (no adjustment) and the number of primary outcome comparisons (full Bonferroni adjustment)" will give an adjustment that will come closer to the 'true' statistical significance level than the 'extreme thresholds' in a majority of situations. Therefore, adjusted threshold was computed for the study from the formula: $\alpha \div [(m+1)/2]$, where 'α' is the unadjusted threshold for significance, usually 0.05 and 'm' is the number of primary outcomes used in the same cohort (in this case: two). Threshold for significance = $0.05 \div [(2+1)/2] = 0.033$. Therefore, a $p< 0.033$ was set as level for statistical significance for the primary outcomes. The secondary outcomes were essentially descriptive; therefore, no adjustment for multiplicity was made.

2.6 Ethical considerations

A copy of the study proposal was submitted to the Human Research Ethic Committees of University of Witwatersrand, Johannesburg, University of Abuja Teaching Hospital and National Hospital, Abuja, for review and approval before the study commenced (see appendix 1). Clearance certificate was also obtained from the National Agency for Food and Drug Administration and Control (NAFDAC) and the study was registered with the Pan African Clinical Trial Registry. Written consent/assent was obtained from the children and their parents/principal guardians before their enrolment into the study. The principles of confidentiality, trust, no-harm and transparency were observed throughout the study. Participation in the study was voluntary.

All participants were thoroughly educated on the nutritional conditions associated with sickle cell crisis and the importance and benefits of the study. The discomfort in the collection of blood specimens, electrocardiography and echocardiography was also explained to the participants.

The participants had no financial obligation, whatsoever, and were given the option to withdraw from participating anytime without needing to give reasons or explanations.

The study was integrated into the existing healthcare system with no attempt to alter the existing management protocol. The results were communicated back to the stakeholders in the child's care.

2.7 Data storage

Study data were captured and stored in REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Witwatersrand, Johannesburg.[131,132] REDCap is a secure, web-based software platform designed to support data capture for research studies, and capable of providing an intuitive interface for validated data capture; it also offer audit trails for tracking data manipulation and export procedures, and an automated export procedures for seamless data downloads to common statistical packages.

CHAPTER 3:

CARDIOVASCULAR CHANGES IN SICKLE CELL CRISIS

This chapter describes and discusses the results of the search for cardiovascular changes in sickle cell crisis.

Most of the content of this chapter have been published in the journal, Cardiology in the Young as:

Richard Onalo, Peter Cooper, Antoinette Cilliers, Uche Nnebe-Agumadu. Cardiovascular Changes in Children with Sickle Cell crisis. *Cardiology in the Young* 2020; **30**: 162-70**.** (IF 0.84)

A preliminary report on impact of sickle cell crisis on blood pressure was presented at the 2018 Union of African Paediatric Societies and Associations as:

Richard Onalo, Uche Nnebe-Agumadu, Oniyangi OO, Antoinette Cilliers, Peter Cooper. Effects of acute painful crisis on blood pressures of children with sickle cell diease. Oral presentation at the joint 14th triennial congress of Union of National African Paediatric Societies and Associations & 49th Annual General and Scientific Conference of Paediatric Association of Nigeria, 24th-26th January, 2018, Abuja, Nigeria.

Part of the study was accepted for Oral presentation at the Nigerian Society of Paediatric Cardiology Scientific Conference, Ibadan, Nigeria, March 22-23, 2020 as:

Onalo R. Cardiac biomarkers in children with sickle cell crisis.

The conference was however terminated due to the Covid-19 pandemic.

3.1 Results

General characteristics

A total of 176 children were recruited, comprising of 92 steady state patients and 84 patients in crisis. The age at presentation was similar for both groups: 10.4±3.2 years for steady state and 10.5±3.4 years for those in crisis, p >0.999. The male: female ratio was 1.2:1 for steady state and 1.3:1 for those in crisis. The average steady state haemoglobin concentration was 7.9 g/dl and 7.7 g/dl, respectively for patients recruited without crisis and those recruited at the time of crisis, p = 0.980. The mean number of blood transfusion in the preceding one year was 0.5±0.7 [median =1 (interquartile range {IQR}, 0-2)] for patients in steady state and 0.5±0.5 [median =1 (IQR, 0-1)] for those enrolled during crisis. The mean (±SD) lifetime blood transfusion was also similar in both study groups: 1.2±1.7 [median =0 (IQR, 0-1)] for patients in steady state and 1.3±1.4 [median =1(IQR, 1-2)] for those in crisis, p = 0.347. Details of the general characteristics are shown in Table 3.1

Table 3.1: Baseline characteristics of patients recruited in steady state and during crisis

Characteristics	**Steady state**	**Crisis**	***P***
Age at presentation	10.39±3.19	10.51±3.43	>0.999
Gender	**N = 92**	**N = 84**	
Male	41(44.56%)	47 (55.95%)	0.179
Female	51(55.43%)	37 (44.05%)	
Social economic class**	**N= 92**	**N = 81**	
Upper class	25 (27.17%)	26 (32.10%)	0.778
Middle class	28 (30.43%)	23 (28.40%)	
Lower class	39 (42.39%)	32 (39.50%)	
№ of crises in the preceding year	**N = 87**	**N= 76**	
None	15 (17.24%)	5 (6.76%)	0.265
1-2	27 (31.03%)	21(28.38%)	
3-6	39 (44.83%)	44 (56.76%)	
>6	6 (6.52%)	6 (7.90%)	
Mean, SD	2.77± 2.51	3.38± 2.35	
Median (IQR)	3 (1-4)	3 (2-4)	
№ of admissions in the preceding year	**N = 88**	**N = 76**	
None	34 (38.64%)	6 (7.89%)	
Once	36 (40.90%)	40 (52.62%)	0.481
Twice	9 (10.23%)	21 (27.63%)	
Three times	7 (7.95%)	6 (7.89%)	
≥ 4 times	2 (2.27%)	3 (3.95%)	
Mean ±SD	0.94± 0.96	1.53± 1.14	
Median (IQR)	1 (0-1)	1 (1-2)	
№ of transfusion in preceding year	**N = 88**	**N = 76**	
None	59 (67.1%)	34 (44.7%)	
Once	24 (27.3%)	40 (52.6%)	0.242
Twice	4 (4.6%)	2 (2.6%)	
≥3	1 (1.14%)	0	
Median (IQR)	1 (0-1)	1 (0-2)	
Lifetime blood transfusion	**N = 88**	**N = 76**	
None	41 (46.6%)	15 (19.4%)	
Once	21 (23.9%)	38 (50.0%)	0.347
Twice	15 (17.1%)	14 (18.4%)	
three times	5 (5.7%)	2 (2.74%)	
≥4 times	6 (6.8%)	7 (8.21%)	
Mean ±SD	1.15± 1.69	1.32± 1.36	
Median (IQR)	0 (0-1)	1 (1-2)	
Weight (kg)	30.67±11.21	30.83±12.57	>0.999
Height (cm)	138.69±15.67	136.82±17.79	>0.999
Body Mass Index	15.50±2.93	15.14±3.27	>0.999
Body Surface Area (m^2)	1.075±0.22	1.06±0.27	0.739

***Social class determined by Oyedeji's method based on father's profession and mother's educational level*[133]

Reproducibility of cardiac parameters assessment

Table 3.2 shows the Bland-Altman agreement analysis of indices of ventricular function and pulmonary haemodynamics. The echocardiographic measurements showed minor intra-observer difference between the online and offline measurements. Mean difference in selected cardiac parameters were narrow. The mean difference in tricuspid regurgitation velocities, for instance, was 0.034 m/s (CI, -0.04 to 0.104) (r = 0.061, p = 0.798) with a 95% limits of agreement of -0.282 to 0.350.

Table 3.2: Intra-observer reproducibility of cardiac parameters measurements

Cardiac Parameters	Mean difference	95% CI of mean difference	Rho	P	95% limits of agreement
TRV	0.034	-0.04, 0.108	-0.061	0.798	-0.282, 0.350
PRV	0.071	-0.144, 0.002	0.088	0.711	-0.384, 0.242
LVIDD	0.062	-0.126, 0.249	-0.102	0.670	-0.739, 0.862
LVIDS	0.018	-0.206, 0.170	-0.073	0.761	0.821, 0.785
IVSD	-0.006	-0.093, 0.082	0.454	0.044	-0.378, 0.367
IVSS	0.058	-0.015, 0.131	0.046	0.848	-0.253, 0.369
LVPWTD	-0.002	-0.059, 0.054	0.428	0.060	-0.243, 0.238
LVPWTS	0.116	-0.010, 0.223	0.220	0.351	-0.340, 0.573
MAPSE	0.042	-0.124, 0.040	-0.007	0.977	-0.390, 0.306
TAPSE	0.027	-0.079, 0.094	-0.090	0.705	-0.363, 0.378
EF	-0.100	-1.315, 1.116	-0.308	0.186	-5.292, 5.093
FS	0.195	-1.941, 1.321	-0.002	0.387	-2.990, 3.380

TRV, tricuspid regurgitation velocity; PRV, pulmonary regurgitation velocity; LVIDD, left ventricular internal dimension in diastole; LVIDS, left ventricular internal dimension in systole; IVSD, interventricular septum in diastole; LVPWTD, left ventricular posterior wall thickness in diastole; LVPWTS, left ventricular posterior wall thickness in systole; MAPSE, mitral annular plane systolic excursion; TAPSE, tricuspid annular plane systolic excursion; EF, left ventricular ejection fraction; FS, left ventricular fractional shortening.

Heart rate and blood pressure changes in crisis

The mean heart rate in patients with crisis [98.1±14.2 bpm (95% CI, 95.2-101.0)] was higher than in those in steady state [86.9±1.4 bpm (95% CI, 86.6-87.2)], ($p < 0.001$). The systolic blood pressures (SBP), diastolic blood pressures (DBP) and the mean arterial blood pressures (MAP) were significantly higher in the crisis group than in the steady state group [SBP mean (±SD), 117.33±14.12 mmHg during crisis vs. 105±9.73 mmHg during steady state, $p < 0.001$; DBP mean (±SD), 71.70±13.32 mmHg in crisis vs. 66.35±8.75 mmHg, $p < 0.01$; MAP mean (±SD), 87.48±13.48 mmHg during crisis vs. 80.53±9.83 mmHg at steady state, $p < 0.001$). Sickle cell crisis is thus associated with a 12.08 ±1.8 mmHg and 5.35±1.69 mmHg increases in systolic and diastolic blood pressures, respectively. The effect of crisis on blood pressures is further displayed in Figure 3.1. During crisis, 40.2% had a systolic blood pressure greater than the 95th percentile (of whom, 21.6% were higher than the 99th percentile +5 mmHg). In the steady state group, markedly elevated blood pressures (>99th percentile +5 mmHg) were not documented. Only 5.4% had a systolic blood pressure greater than the 95th percentile. Thus, elevated SBP is common during crisis but rare during the steady state.

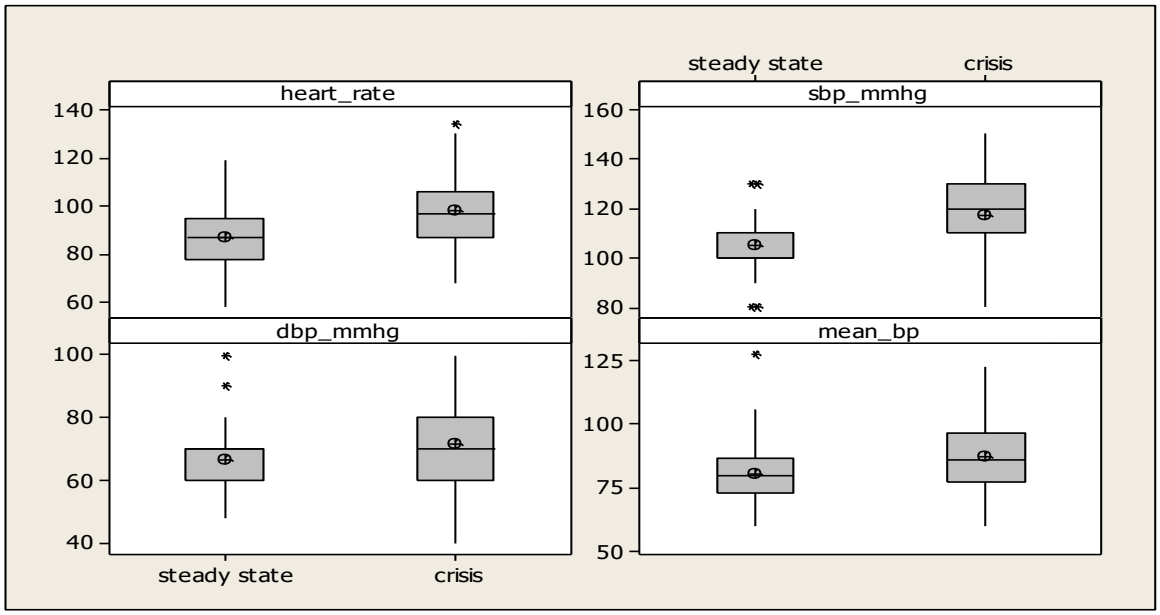


Figure 3.1: Boxplot of heart rate and blood pressure pattern in sickle cell crisis and in steady state showing higher heart rate and blood pressures mean, median and interquartile range in the crisis state compared to the steady state.

Similarly, elevations in the DBP was observed in patients recruited during the crisis state, with 31% of them having a DBP greater than the 95th percentile (19.2% of which were greater than

the 99th percentile +5 mmHg) during crisis, while only 9.6% of those in steady state had blood pressure in excess of the 95th percentile. None was greater than the 99th percentile.

Prevalence of conduction abnormalities

The prevalence of prolonged PR interval [5.4% (95% CI, 0.6-10.2)] and prolonged corrected QT interval [32.6% (95% CI, 22.5-42.6)] during the steady state was about half the prevalence in crisis [13.3% (95% CI, 6.4-20.2) for prolonged PR interval, p = 0.075] and [55.4% (95% CI, 45.2-65.6) for prolonged corrected QT interval, p = 0.002], Figure 3.2. Sickle cell crisis doubled the prevalence of interventricular conduction delay, from 7.6% (95% CI, 2.2-13.0) in the steady state group to 14.5% (95% CI, 7.0-22.0) in the crisis group, even though, the increase failed to reach statistical significance, p = 0.142.

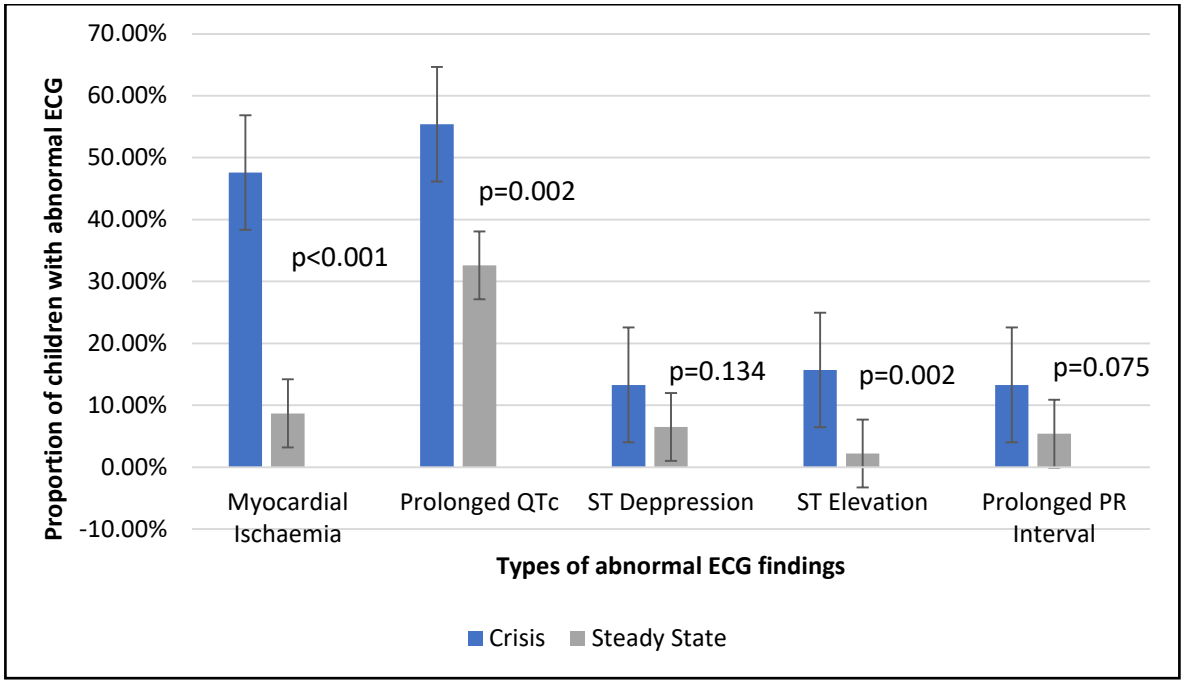


Figure 3.2: Influence of sickle cell crisis on the proportion of children with prolonged PR, QTc, ST segment changes and significant myocardial ischaemia scores

Indices of myocardial ischaemia

The prevalence of ST segment elevation was increased 7-fold by sickle cell crisis: 2.2% (95% CI, -0.9-5.3) for patients in steady state, vs. 15.7% (95% CI, 8.3-23.1), for patients in crisis, p =

0.002 as shown in Figure 3.2. The increase in prevalence of ST depression, however, failed to reach statistical significance, p = 0.134. The mean myocardial ischaemia score (a composite of T wave abnormalities, ST segment changes and conduction abnormalities) obtained in patients having crisis [3.14±1.61 (95% CI, 2.80-3.45)] was higher than that recorded in patients recruited at steady state [1.97±1.41 (95% CI, 1.66-2.28)], p <0.001. Among patients having crisis, those with acute chest syndrome (n = 11) had higher mean ischaemia score than those without acute chest syndrome (n = 73): 4.4±1.59 versus 3.33±1.28, p = 0.02. Mean myocardial ischaemia score had a negative correlation with haemoglobin concentration at presentation (r = -0.21, p = 0.013), oxygen saturation (r = -0.30, p < 0.001), and systemic vascular resistance (r = -0.30, p < 0.001), but correlated positively with number of blood transfusion in the preceding year, pulmonary artery pressures, right ventricular Tei index and left ventricular wall thickness as shown in Table 3.3. Using an MI score of >3 as diagnostic criteria, myocardial ischaemia was present in 8 (8.7%) of the 92 patients in steady state and 40 (47.6%) of the 84 patients in crisis, thus indicating an increase in MI prevalence by 38.9% in crisis state.

Table 3.3.: Correlation of myocardial ischemia score with clinical and echocardiographic parameters

Parameters	**R**	**p value**
№ of admission in the preceding year	0.14	0.077
№ of blood transfusion in the preceding year	0.20	**0.010**
Lifetime blood transfusion	0.19	**0.017**
Haemoglobin concentration at presentation	-0.21	**0.013**
Systolic Blood Pressure	0.29	**<0.001**
Diastolic Blood Pressure	0.13	0.056
Mean Blood Pressure	0.20	**0.004**
Oxygen saturation	-0.30	**<0.001**
Corrected-QT interval	0.47	**<0.001**
Tricuspid Regurgitation Velocity	0.38	**<0.001**
Pulmonary Regurgitation Velocity	0.23	**0.001**
Pulmonary Artery Systolic Pressure	0.44	**<0.001**
Pulmonary Artery Diastolic Pressure	0.35	**<0.001**
Mean Pulmonary Artery Pressure	0.45	**<0.001**
Left Ventricular end-diastolic Dimension z score	0.14	0.052
Left Ventricular Posterior Wall Thickness z score	0.26	**0.002**
Mitral Annular Plane Systolic Excursion z score	0.15	**0.029**
Left Ventricular Tei Index	0.07	0.339
Right Ventricular Tei Index	0.16	**0.025**
Tricuspid Annular Plane Systolic Excursion z score	0.14	0.054
Pulmonary Vascular Resistance	0.17	**0.013**
Systemic Vascular Resistance	-0.30	**<0.001**

Echocardiographic changes detected during crisis in the right and left heart

Both tricuspid and pulmonary regurgitation velocities as well as pulmonary artery pressures and pulmonary vascular resistance calculations were elevated during crises. Similarly, the mean circumferential wall stress index in patients having crisis (218.0±59.5 g/cm^2) was higher than the value recorded for steady state patients (195.1±44.9 g/cm^2), p = 0.008, but there was no statistically significant difference in the meridional wall stress index between the two groups: 79.2±23.4 g/cm^2 (steady state) versus 88.5±30.8 g/cm^2 (crisis group), p = 0.055 (Table 3.4). The left ventricular fractional shortening (35.7±5.0%) and ejection fraction (64.8±6.8%) mean values in steady state were comparable to those obtained during crisis state: 37.1±5.3% and 66.6±7.0%, respectively.

Table 3.4: Comparison between echocardiographic parameters of patients in steady state and of those in crisis

Echocardiographic Indices	**Steady state Mean**±SD	**Crisis Mean**±SD	**p value**
Tricuspid regurgitation (m/s)	1.52±0.84	2.17±0.93	**<0.001**
Tricuspid regurgitation gradient (mmHg)	12.02±8.49	22.26±11.72	**<0.001**
Pulmonary regurgitation velocity (m/s)	1.05±0.62	1.28±0.77	0.094
Pulmonary regurgitation gradient (mmHg)	5.95±5.06	8.73±7.76	**0.010**
Pulmonary artery systolic pressure (mmHg)	22.71±8.81	33.77±12.12	**<0.001**
Pulmonary artery diastolic pressure (mmHg)	16.65±5.29	20.62±8.36	**<0.001**
Mean pulmonary artery pressure (mmHg)	18.67±5.16	25.00±7.78	**<0.001**
Pulmonary vascular resistance (Wu)	0.92±0.43	1.44±1.44	**0.002**
Pulmonary capillary wedge pressure (mmHg)	8.45±2.56	8.26±1.44	>0.999
Meridional Wall stress index (g/cm^2)	79.2±23.4	88.5±30.8	0.055
Circumferential Wall stress index (g/cm^2)	195.1±44.9	218.0±59.5	**0.008**
Systemic vascular resistance (Wu)	18.45±5.36	18.13±6.18	>0.999
Left ventricular ejection fraction (%)	64.8±6.8	66.6±7.0	>0.999
Left ventricular fractional shortening (%)	35.7±5.0	37.1±5.3	>0.999
Ratio of right ventricular systolic pressure to systemic systolic pressure	0.22±0.09	0.30±0.10	**<0.001**

Prevalence of ventricular dysfunction and pulmonary hypertension

Using a left ventricular Tei index >0.45, the prevalence of left ventricular dysfunction was 2.5-fold higher in patients having crisis than in patients without crisis: 16.7% versus 6.5%, p = 0.03 (Figure 3.2 and Table 3.5). Likewise, the number of children with mitral annular plane systolic excursion z score <-2 was 5-times higher in the crisis group than in the steady state group: 13 (15.5 %) versus 3 (3.3%), p = 0.01. Furthermore, the biochemical markers of left ventricular dysfunction were higher among patients having sickle cell crisis than in steady state, p<0.01. On the other hand, the prevalence of right ventricular dysfunction (right ventricular Tei index value >0.4) was 3 times higher in the crisis group than in the steady state group (3.3% in steady state versus 10.7% in crisis). This difference, however, fell short of statistical significance, p = 0.052. (Figure 3.3 and Table 3.5).

The prevalence of pulmonary hypertension (mean pulmonary artery pressure >25mmHg) tripled among those with sickle cell crisis when comparing them with those in steady state (47.6% vs. 14.1%).

Table 3.5: Comparison of the prevalence of cardiac dysfunction and pulmonary hypertension in steady state and in crisis

Index of cardiac dysfunction and pulmonary hypertension	**Number of patients, n (%)**		
	Steady state	**Crisis**	**p value**
Left Ventricular Ejection fraction <55%	6(6.52)	8(9.52)	0.462
Left Ventricular Fractional shortening <28%	6(6.52)	4(4.76)	0.610
Left Ventricular Tei index >0.45	6(6.52)	14(16.67)	**0.034**
Mitral Annular Plane Systolic Excursion Z score <-2	3(3.26)	13(15.48)	**0.005**
Right Ventricular fractional area change <35%	14(15.22)	16(19.05)	0.500
Right Ventricular Tei index >0.40	3(3.26)	9(10.71)	0.052
Tricuspid Annular Plane Systolic Excursion Z score <-2	9(9.78)	13(15.48)	0.250
Tricuspid regurgitation velocity >2.5m/s	6(6.52)	43(51.19)	**<0.001**
Tricuspid regurgitation gradient >30mmHg	0	24(51.19)	**<0.001**
Pulmonary artery systolic pressure >40mmHg	1(1.09)	30(35.71)	**<0.001**
Mean pulmonary artery pressure >25mmHg	13(14.13)	40(47.62)	**<0.001**
N-Terminal pro B-type Natriuretic Peptide >160pg/mL	13(14.13)	33(38.82)	**<0.001**
Cardiac Troponin I > 0.2ng/mL	6(6.52)	23(27.07)	**<0.001**

The interval plot of systemic vascular resistance, pulmonary vascular resistance and wall stress index mean values are shown in Figure 3.3. The interaction tests for systemic vascular resistance ($p > 0.99$) and wall stress index [(meridional ($p = 0.373$) and circumferential ($p = 0.21$)] were not significant, whereas the interaction test for pulmonary vascular resistance in steady state and crisis was significant ($p = 0.002$).

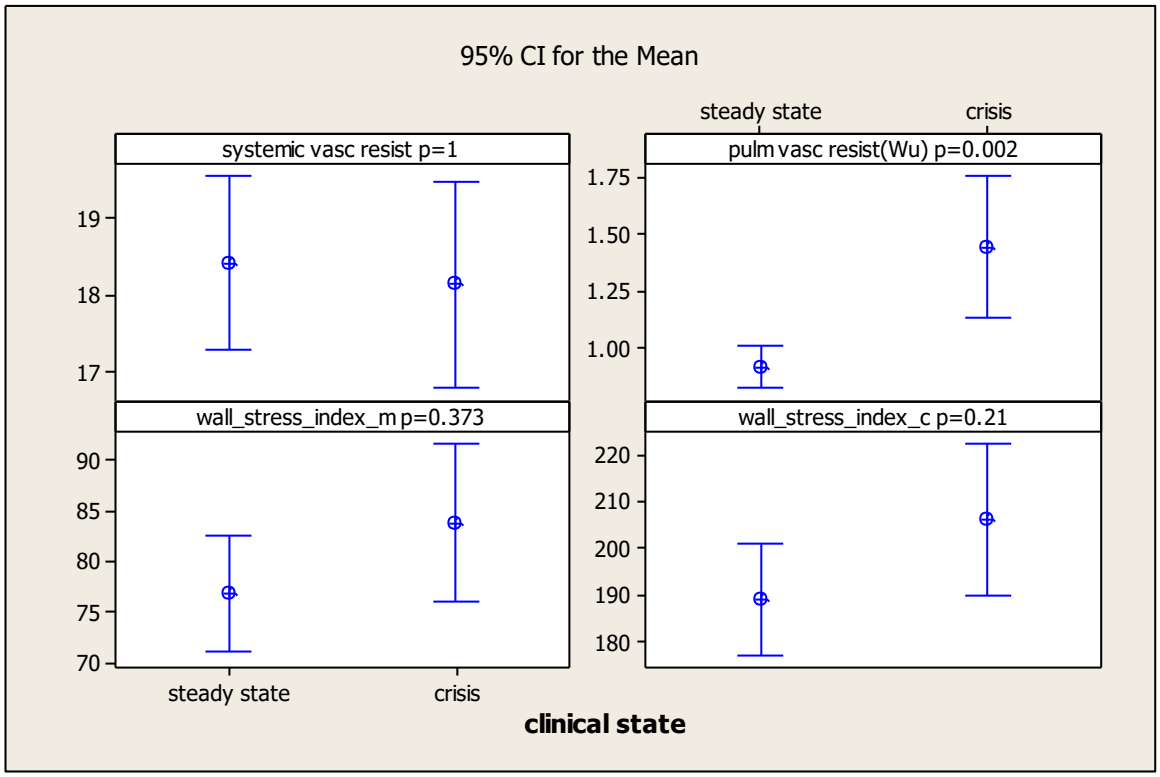


Figure 3.3: Interval plot of systemic vascular resistance, pulmonary vascular resistance and wall stress index in steady state and in crisis showing an interaction test p-value of 0.002 for pulmonary vascular resistance (pulm vasc resist). The confidence interval for systemic vascular resistance (systemic vasc resist) and wall stress index were similar for patients in steady state and in crisis. M = meridional, C = circumferential.

Effects of sickle cell crisis on cardiac biomarkers

The median NT-proBNP level was 6.00 pg/ml (IQR, 2.00-74.93) for patients in steady state and 73.3pg/ml (IQR, 5.2-273.8) in individuals with sickle cell crisis. Thus, sickle cell crisis is associated with increase in plasma NT-proBNP, $p < 0.005$. Similarly, the interval plot of 95% Confidence Interval for mean showed that the difference between the NT-proBNP in patients who had crisis and those in steady state was significant, with interaction test $p < 0.05$ (Figure 3.4).

The median troponin I level was 0.035 ng/ml (IQR, 0.01-0.055) for patients in steady state but almost doubled in patients that had crisis: 0.063ng/ml (IQR, 0.017-0.212), $p = 0.031$

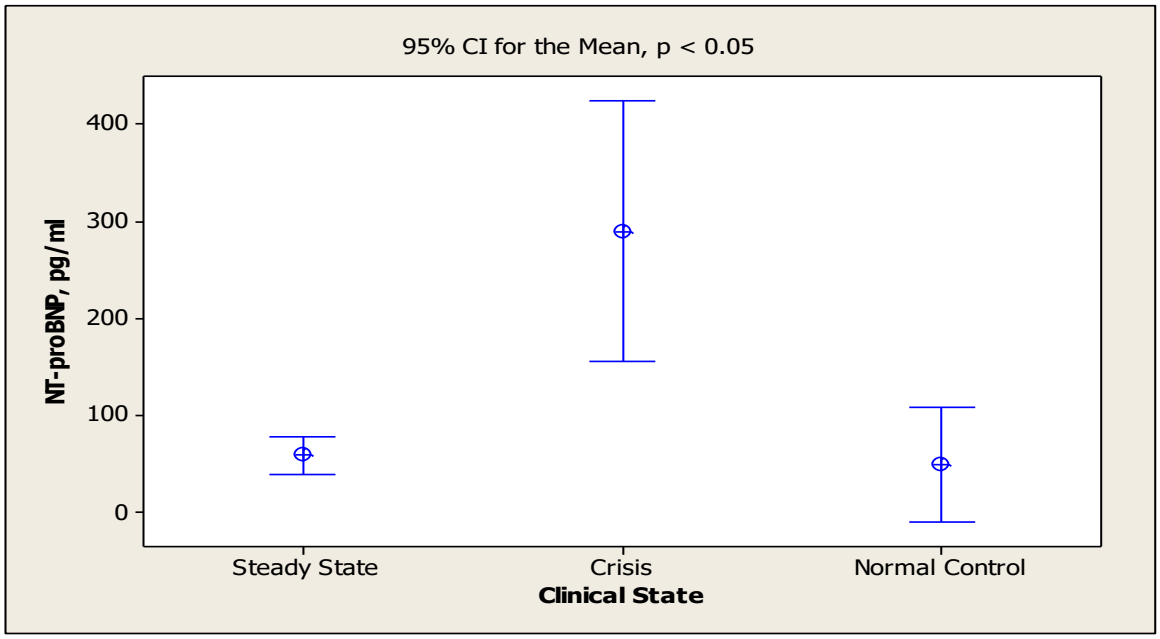


Figure 3.4: Interval plot of mean values of NT-proBNP in sickle cell anaemia patients in steady state and in crisis along with those of normal controls. The bars represent 25th and 75th percentile, while the star represent the median, which is higher in patients having crisis

Relationship between cardiac biomarkers and electrocardiographic indices of myocardial ischaemia

Prolongation of the PR interval occurred with similar prevalence among patients with high and normal cardiac biomarkers. Prolonged corrected-QT interval was however, associated with elevated troponin I ($p = 0.013$) and NT-proBNP ($p = 0.001$) levels. As shown in Table 3.6, ST segment changes were more common in patients with elevated NT-proBNP ($p = 0.030$), but occurred with similar proportions in patients with high as well as normal troponin I levels. Pathologically inverted T waves were more common in patients with elevated troponin I and NT-proBNP, though the frequency of occurrence fell short of statistical significance.

The prevalence of high myocardial ischaemia score, a composite of PR prolongation, QTc prolongation, T wave inversion and ST segment changes, was higher in patients with high troponin I levels ($p = 0.016$) and high NT-proBNP ($p = 0.001$).

Table 3.6: Electrocardiographic changes in relation to cardiac troponin I and NT-proBNP levels

ECG Variables	Trop I <0.2 N=146	Trop I ≥0.2 N=30	p-value	NT-proBNP <160pg/ml N=126	NT-proBNP ≥160pg/ml N=50	p-value
Prolonged PR Intervals	14 (9.6)	2 (6.7)	0.463	11 (8.7)	4 (8.0)	>0.999
Prolonged QTC Intervals	52 (35.6)	18 (60.0)	**0.013**	50 (39.7)	34 (68.0)	**0.001**
ST changes	26 (17.8)	7 (23.3)	0.480	21 (16.7)	14 (28.0)	**0.030**
T inversion >3 chest leads	83 (56.8)	19 (63.3)	0.512	65 (51.6)	31 (62.0)	0.211
Flat/biphasic T ≥2 chest leads	10 (6.8)	1 (3.3)	0.690	14 (11.1)	4 (8.0)	0.539
Myocardial Ischaemia score >3	67 (45.9)	21 (70.0)	**0.016**	53 (42.1)	35 (70.0)	**0.001**

Cardiac biomarkers and echocardiographic indices of cardiac haemodynamics

There was a weak positive correlation between mean pulmonary artery pressure and NT-proBNP levels, r = 0.2259, p = 0.039). Patients with high NT-proBNP levels (≥160 pg/ml) had higher pulmonary regurgitation jet velocity, p = 0.033, higher pulmonary artery systolic pressure (31.86±13.02 mmHg vs. 23.64±11.91 mmHg in patients having normal NT-proBNP, p = 0.001), higher pulmonary artery diastolic pressure (20.39±9.21 mmHg vs. 16.38±6.39 mmHg recorded in patients with normal NT-proBNP, p = 0.001) and higher mean pulmonary artery pressures (24.21±8.72 mmHg as against 18.79±7.19 mmHg in patients with normal NT-proBNP, p < 0.001). There was however no relationship between high NT-proBNP and pulmonary capillary wedge pressure (p > 0.999) and pulmonary vascular resistance (p = 0.343) (Table 3.7).

Table 3.7: Relationship between NT-prohormone BNP levels and cardiac haemodynamics

Cardiac hemodynamics	**NT-pro BNP Levels**		**95% CI of diff in mean**	**p-value**
	<160(n=126)	**≥160(n=50)**		
Tricuspid regurgitation velocity, m/s	1.70±2.37	2.06±0.95	-0.32, 1.04	0.299
Pulmonary regurgitation velocity, m/s	1.04±0.67	1.26±0.81	-0.01, 0.45	**0.033**
Pulmonary artery systolic pressure, mmHg	23.64±11.91	31.86±13.02	4.18, 12.26	**0.001**
Pulmonary artery diastolic pressure, mmHg	16.38±6.39	20.39±9.21	1.60, 6.42	**0.001**
Mean Pulmonary artery pressure, mmHg	18.79±7.19	24.21±8.72	2.89, 7.94	**<0.001**
Left Ventricular Mass Volume Index	2.77±4.46	2.73±1.22	-1.31, 1.23	0.950
Left Ventricular Tei Index	0.31±0.12	0.33±0.16	-0.02, 0.06	0.368
Right Ventricular Tei Index	0.22±0.099	0.27±0.10	0.02, 0.08	**0.002**
Meridional wall stress index	89.25±29.23	83.78±33.00	-4.54, 15.48	0.282
Circumferential wall stress index	191.36±60.40	201.01±75.60	-11.81, 31.11	0.379
Pulmonary Vascular Resistance, Wu	1.04±1.14	1.20±0.52	-0.17, 0.49	0.342
Pulmonary Capillary Wedge Pressure, mmHg	8.34±1.66	8.34±2.097	-0.59, 0.59	>0.999

Cardiac troponin levels were generally low (<0.2 ng/ml) in 71.6% of the patients. Cardiac troponin I levels of ≥0.2 ng/ml were found in patients with high pulmonary artery systolic pressure (32.72±11.91 mmHg vs. 24.43±2.42 mmHg in patients with normal troponin I levels, $p = 0.001$), high pulmonary artery diastolic pressures (21.54±9.24 mmHg vs. 16.64±6.79 mmHg in patients with normal troponin I, $p = 0.001$), and high mean pulmonary artery pressure (25.26±8.49 mmHg vs. 19.23±7.49 mmHg in patients with normal troponin I, $p < 0.001$). Again, the pulmonary capillary wedge pressure ($p = 0.5302$) and pulmonary vascular resistance ($p = 0.353$) showed no association with troponin I levels. The NT-proBNP level showed tendency to increase with increase in myocardial ischemia score values ($r = 0.2449$, $p = 0.019$), especially during crisis.

Table 3.8: Association of cardiac troponin I levels with cardiac haemodynamics

Cardiac hemodynamics	**Cardiac troponin I levels, ng/ml**		**95% C1 of diff in mean**	**p value**
	<0.2 (n = 146)	≥0.2 (n=30)		
Tricuspid regurgitation velocity, m/s	1.73±2.26	2.10±0.89	-0.45, 1.20	0.379
Pulmonary regurgitation velocity, m/s	1.06±0.68	1.27±0.88	-0.07, 0.49	0.146
Pulmonary artery systolic pressure, mmHg	24.43±2.42	32.72±11.91	3.41, 13.17	**0.001**
Pulmonary artery diastolic pressure, mmHg	16.64±6.76	21.54±9.24	2.038, 7.76	**0.001**
Mean Pulmonary artery pressure, mmHg	19.23±7.49	25.26±8.49	2.99, 9.06	**<0.001**
Left Ventricular Mass Volume Index	2.77±4.46	2.92±1.43	-1.48, 1.78	0.856
Left Ventricular Tei Index	0.31±0.11	0.31±0.16	-0.05, 0.05	>0.999
Circumferential wall stress index	194.46±65.62	189.23±57.14	-30.66, 20.20	0.685
Meridional wall stress index	79.01±32.24	76.02±27.37	-15.44, 9.46	0.636
Pulmonary Capillary Wedge Pressure, mmHg	8.38±2.07	8.13±1.47	-1.03, 0.53	0.530
Pulmonary Vascular Resistance, Wu	1.05±1.09	1.24±0.53	-0.21, 0.59	0.353
Right Ventricular Tei Index	0.22±0.10	0.26±0.10	-3.57, 1.58	0.983
Myocardial Ischaemia Score	2.10±1.43	3.17±1.87	2.16, 2.65	**<0.001**

Correlation between indices of cardiac function and cardiac biomarkers

The RV and LV Tei index showed weak and strong positive correlations, respectively with NT-proBNP levels (r = 0.220, p = 0.036, RV Tei Index), (r = 0.998, p = 0.045, LV Tei Index) especially during crisis.

The mean RV Tei index was 0.27±0.10 in patients with NT-proBNP levels of ≥160 pg/ml as against 0.22±0.10 (95% CI of 0.02 to 0.08) in the group with NT-proBNP of less than 160 pg/ml, p = 0.002. Left ventricular Tei index tended to be higher (0.33±0.16) in patients with NT-proBNP ≥160 pg/ml than in patients with NT-proBNP levels of less than 160 pg/ml (0.31±0.12) with a 95% CI of -0.02 to 0.06, p = 0.368.

Cardiac biomarkers levels in specific sickle cell crisis situations

Cardiac biomarker levels are elevated in sickle cell crisis, but the elevation was more pronounced in certain types of crisis.

The highest troponin I level occurred in patients with acute chest syndrome, median of 1.243 ng/ml (IQR, 0.05-3.34), followed by those with mixed crisis comprising of acute chest syndrome and vaso-occlusive bone pain crisis, median = 0.31 ng/ml, (IQR, 0.05-1.277), and a combination of vaso-occlusive crisis with anaemic crisis, median = 0.13 ng/ml (IQR, 0.08-0.205), Table 3.9.

NT-proBNP levels on the other hand were highest in patients with a combination of acute chest syndrome and vaso-occlusive crisis: median of 395 pg/ml (IQR, 297-1465), followed by the coexistence of vaso-occlusive and anaemic crisis: median = 303 pg/ml (IQR, 76-809). For those with purely defined single crisis, acute chest syndrome had the highest NT-proBNP levels [median = 1546 pg/ml (IQR, 57-2025)].

Table 3.9: Cardiac biomarker levels in various sickle cell crisis states

Types of crisis	**Troponin I levels**	**NT-proBNP levels**
	Median (IQR) ng/ml	Median (IQR) pg/ml
No crisis (n=92)	0.03 (0.11 – 0.05)	6.6 (2.0-55.7)
Acute cerebral syndrome (n=4)	0.02 (0.01 – 0.49)	44.9 (12.3-231.0)
Vaso-occlusive bone pain crisis (n=54)	0.05 (0.012 – 0.203)	45.0 (2.0-195.7)
Anaemic crisis (n=3)	0.034 (0.010 – 0.038)	68.2 (2.0-185.1)
Acute chest syndrome (n=3)	1.243 (0.05 – 3.34)	154 (57-2025)
Mixed crisis (VOC+ Anaemic) (n=9)	0.13 (0.08 – 0.205)	303 (76-809)
Mixed crisis (VOC + ACS) (n=6)	0.31 (0.05 – 1.277)	395 (297-1465)

3.2 DISCUSSION

Sickle cell crisis triggers a cascade of events in the cardiovascular system, including increases in heart rate, blood pressure, and electrical and functional abnormalities. In this study, the heart rate was higher in those in crisis compared with those in steady state, by an average of 12 beats per minute, a finding corroborated by the reports of other authors.[134,135] Many factors in crisis acting independently and/or cumulatively could increase the heart rate, including pain, fever, hypoxia and myocardial ischaemic injury. Tachycardia as a compensatory mechanism can cause an increase in oxygen consumption with a possible worsening of myocardial ischaemia.

Sickle cell patients generally have lower blood pressures than individuals with normal haemoglobin genotype (hemoglobin AA).[136,137] However, a transient elevation in systemic blood pressures has been documented in sickle cell crisis.[134,135] An average difference of 14 mmHg in systolic pressure was observed between children in crisis and those in steady state. Of those in crisis, 40.2% had systolic blood pressure greater than the 95th percentile, of whom, 21.6% were higher than the 99th percentile plus 5 mmHg. In contrast only 5.4% of children in the steady state were documented to have systolic blood pressure greater than the 95th percentile and none was found to have blood pressure greater than the 99th percentile plus 5 mmHg in the present study. Elevations in diastolic blood pressure were also observed, with 31% of those in crisis having diastolic blood pressure greater than the 95th percentile, 19.2% of which were greater than the 99th percentile plus 5 mmHg. A possible explanation for the elevation of blood pressure in sickle cell crisis is the marked increase in total blood volume and cardiac output during crisis.[138-140] Acute pain causes elevation of blood pressure by direct stimulation of the sympathetic (autonomic) pathway by electrical pain signals reaching the central nervous system, and by activation of the neuroendocrine pathway particularly the hypothalamic–pituitary–adrenal axis causing the release of epinephrine and other catecholamine from the adrenal medulla. These metabolites cause vasoconstriction of the peripheral blood vessels and also induce a positive chronotropic and ionotropic effect on the myocardium, thus increasing the cardiac output and raising the blood pressure. Acute elevations of blood pressures in excess of the 99th percentile plus 5 mmHg may alter the regulation of the blood brain barrier resulting in cerebral hyper-perfusion syndromes which manifest with symptoms such as headaches. Furthermore, children with very high blood pressures have been shown to be at-risk for silent strokes and associated memory loss. The

same report showed the relative risk of silent stroke in sickle cell disease children to be 1.7 times those with lower blood pressures.[138] Although, four patients with crisis in our study presented with overt stroke, the role of blood pressure elevation in their stroke could not be determined. An elevated systemic blood pressure during crisis may be associated with the development of left ventricular dysfunction.[141] Although, there is no definitive proof, the elevated blood pressures recorded during crisis could have had a role in the 2-fold increase in the proportion of children with cardiac dysfunction compared to those in a steady state. Further studies are required to confirm this relationship.

Acute increases in pulmonary artery pressures recorded in the presence of severe vaso-occlusive bone pain crisis and acute chest syndromes,[141] is poorly tolerated by adult sickle cell patients.[63] An increase of 10 mmHg in the mean pulmonary artery pressure has been associated with 1.7-times increase in the hazard ratio of death.[142] The acute pressure change exerts pressure overload on the right ventricle which could precipitate right ventricular dysfunction. The proportion of patients with high pulmonary artery pressures and right ventricular dysfunction were higher in the crisis than in the steady state group in this study. About 48% of patients in the crisis state had elevated blood pressure as against the 14% in the steady state, giving a three-fold increase in prevalence of pulmonary hypertension during vaso-occlusive crisis. It is not clear if the increase in prevalence of pulmonary hypertension has any direct relationship with the increase in right ventricular systolic dysfunction in this study. Pressure overload in the right ventricle increases the work of the heart and myocardial oxygen demand, and hence increased tendency to myocardial ischaemia when exposed to hypoxic conditions.

Electrocardiographic evaluations of patients with sickle cell crisis revealed features suggestive of myocardial ischaemia.[57,58] In this study, ST segment abnormalities, particularly ST elevation, and prolonged PR and QT intervals were more prevalent during crisis. However, unlike Maisel *et al*[139] who used 12-24 hours continuous electrocardiographic monitoring, the presence of ventricular arrhythmias in the study patients was not documented, most likely because a single 12-lead electrocardiogram was performed during admission for sickle cell anaemia crisis.

T-wave abnormalities suggestive of myocardial ischaemia (Flat T-waves, T-wave inversion in more than two contiguous leads) have been documented in children with sickle cell crisis in a

study from Nigeria by Bode-Thomas *et al.*[57] The use of the composite myocardial ischaemia score devised by these authors, as well as the addition of cardiac biomarkers further increases the sensitivity and specificity of the diagnosis of myocardial ischaemia. A significant proportion of children with sickle cell crisis in the present study (47.6%) fulfilled the Bode-Thomas' electrocardiographic criteria for myocardial ischaemia. However, the prevalence of myocardial ischaemia was reduced to 40% when elevated myocardial injury biomarkers such as N-terminal pro B-type natriuretic peptides and cardiac troponin I were added as evidence of myocardial injury suggesting the need for caution when using ST-T wave abnormalities alone.

Radionuclide cardiac scans and autopsy results[59] have shown the mechanism of myocardial ischaemia to be due to microvascular occlusion with sickled red blood cells in some patients. The chest pain experienced by certain patients during crisis in the present study may be due to a vaso-occlusive process resulting in myocardial ischaemia. Further evidence of myocardial ischaemia caused by vascular occlusion is the finding of left ventricular wall motion abnormalities by Maisel *et al*[139] during sickle cell crisis in patients presenting with an acute chest syndrome and severe vaso-occlusive bone pain crisis.

Echocardiographic imaging in the present study showed that the prevalence of left ventricular dysfunction (based on left ventricular Tei index >0.45 and mitral annular plane systolic excursion z score <- 2) was about three times higher in patients having crisis than in those in steady state. Furthermore, right ventricular dysfunction (right ventricular Tei index >0.4) is more prevalent during a crisis than in the steady state. Cardiac dysfunction in sickle cell crisis may result from hypoxemia due to pulmonary complications and/or precipitous drop in the packed red cell volume. Furthermore, significant myocardial ischaemia can compromise cardiac function. There was, however, no adverse outcome with regard to the detected left ventricular dysfunction in the present study, thus suggesting that deranged echocardiographic parameters may not necessarily indicate the presence of a clinical disease.

The presence of elevated pulmonary artery pressure during a crisis is reflected by the tricuspid regurgitant jet velocity which was higher (mean of 2.2 m/s) in the crisis group than in the steady state group (mean of 1.52 m/s). Several publications[30,57,59,62,63] have shown up to a fourfold increase in mortality and sudden death in patients with elevated pulmonary pressures during a crisis. In the present study, the mean pulmonary artery pressure in patients with sickle

cell crisis was higher than those of patients in steady state with 47.6% of those in crisis having a mean pulmonary artery pressure greater than 25 mmHg compared to 14.1% in the steady state group. One patient who presented with acute chest syndrome in the current study died suddenly during the crisis before a full echocardiographic and electrocardiographic evaluation could be done. Although, the cause of death is unknown, it may not be unconnected with an acute cardiopulmonary event.

Cardiac biomarkers were elevated in patients recruited during sickle cell crisis, which again, supports the hypothesis that the crisis state is a stressor to an already stretched myocardium.[143] Acute ventricular distensions and myocardial ischaemia may cause the elevation of NT-proBNP and troponin I within the circulation. Takatsuki *et al*[144] corroborated this finding among 33 children hospitalised for vaso-occlusive pain crisis and/or acute chest syndrome in Colorado Children Hospital, Aurora, Colorado. Although the prevalence of elevated troponin I in individuals with sickle cell crisis is relatively low: 6.3% in adults (Baltimore, USA)[145] and 15.9% in children (Jeddah, Saudi Arabia),[146] the comprehensive care of patients with acute chest syndrome and chest pain should include NT-proBNP and troponin I assay in view of the potential of detecting myocardial injury even if it is subclinical.

Various pertinent cardiovascular changes were documented during sickle cell crisis in this study including an elevation in blood pressures, subclinical myocardial ischaemia, reduction in left ventricular systolic performance, an increase in the velocity of the tricuspid regurgitation jet and consequently estimate of the pulmonary artery pressure, and also the elevation of cardiac biomarkers.

3.3 SUMMARY OF MAJOR FINDINGS

The present data suggests that sickle cell crisis has negative effects on the cardiovascular system. The prevalence of systemic hypertension, myocardial ischaemia, and left ventricular dysfunction and evidence of pulmonary hypertension was significantly higher among children with sickle cell crisis. NT-proBNP and cardiac troponin I levels were also significantly increased among patients with crisis compared to their steady state counterparts.

CHAPTER 4:

IMPACT OF ARGININE SUPPLEMENTATION ON CARDIOVASCULAR CHANGES IN VASO-OCCLUSIVE CRISIS AND ACUTE CHEST SYNDROME

This chapter describes the effects of arginine supplementation on altered cardiovascular parameters in sickle cell crisis. The results are provided and discussions based on the findings are outlined.

A manuscript arising from the results has been presented in a scientific conference as:

Onalo R, Cilliers A, Cooper P. Effect of $_{L}$-arginine supplementation on blood pressure of patients with sickle cell crisis. Nigerian Society of Paediatric Cardiology, Ibadan, March, 2020.

Other articles being prepared for publication include:

1. Impact of arginine therapy on the prevalence of cardiovascular dysfunction in sickle cell crisis.
2. Beneficial role of oral $_{L}$-arginine supplementation in children with elevated tricuspid regurgitation velocities.

4.1 RESULTS

Enrolment

The CONSORT flow diagram (Figure 4.1) illustrates the screening, enrolment and follow up of the patients that participated in the study. Of the 66 patients that were randomized, forty-eight (72.7%) received the maximum 15 doses of the treatment or placebo. All the patients that discontinued the drugs did so because they were discharged home.

Baseline characteristics of the study patients

A total of 66 SCA patients were recruited for the study comprising of 38 males and 28 females (M: F=1.36:1). The mean age of the patients was 10.63±0.41 years, range 5-17 years. The age, gender and social class distributions were similar in the arginine and placebo groups (Table 4.1). Similarly, the weight, height, BMI and BSA were comparable in both groups. The median number of crisis in the preceding one year were similar in both groups, as was the number of blood transfusions in the preceding year and lifetime blood transfusion. Steady state Hb concentration and the Hb concentrations on admission were also similar for both groups as shown in Table 4.1.

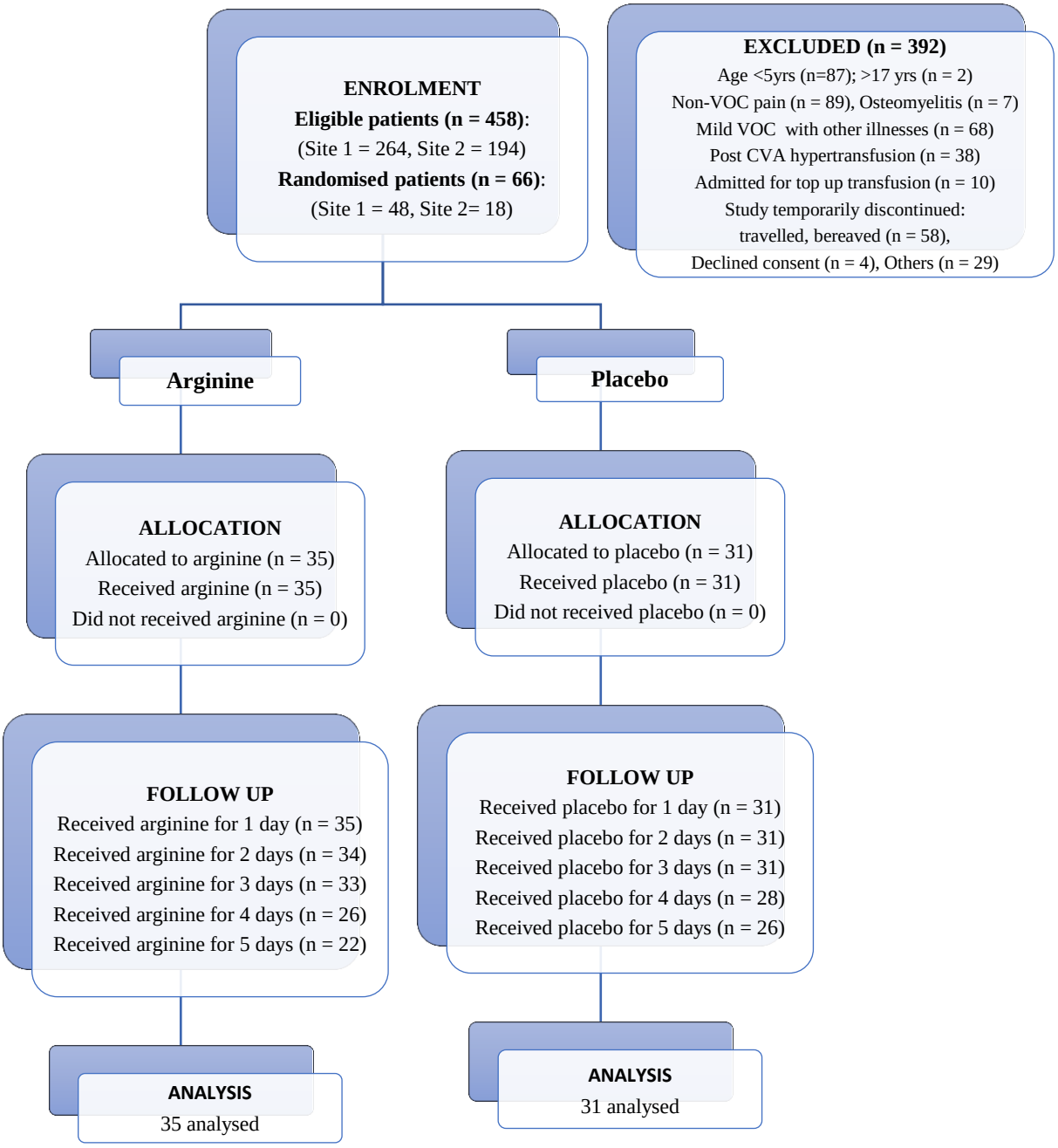


Figure 4.1: CONSORT flow diagram of the enrolment and randomisation process of participants in impact of arginine on cardiovascular changes in crisis
***Other reasons for exclusion: Guardian not around to give consent (n = 3), needed immediate blood transfusion (n = 6), readmission (n = 7), hepatitis (n = 5), treatment commenced before diagnosis was made (n = 5), inadvertently missed(n = 2, acute kidney injury (N =1)

Table 4.1: Baseline characteristics of patients

Parameters	**Arginine group (N=35)**	**Placebo group (N=31)**	**p-value**
Gender n (%)			
Male	18(51.4)	20(64.5)	0.562
Female	17(48.6)	11(35.5)	
Social Class** n (%)			
Upper class	15(42.9)	10(32.3)	
Middle class	10(28.6)	8(25.8)	0.793
Lower class	10 (28.6)	12 (38.7)	
Age Mean±SD (years), range	10.75±3.21, 5-17	10.50±3.55, 5-17	0.858
Weight Mean±SD (kg), 95% CI	30.3±8.30, 17.10-57.80	31.72.±14.49, 15.00-80.00	0.622
Weight z Mean±SD, 95% CI	-1.83±1.92, -8.2-1.99	-1.56±1.84, -5.31-1.99	0.563
Height Mean±SD (cm), 95% CI	139.9±15.55, 110-178	141.7±17.45, 108-176	0.659
Height z Mean±SD, 95% CI	-1.19±1.73, -5.2-3.79	-0.64±1.74, -3.35-4.09	0.203
BMI Mean±SD (kg/m²), 95% CI	15.32±2.51, 11.85-23.67	15.17±3.79, 8.48-27.68	0.849
BSA (m^2) Mean±SD, 95% CI	1.08±0.21, 0.74-1.74	1.10±0.30, 0.67-1.92	0.753
Pulse rate (bpm), 95% CI	101.94, 96.22-107.66	106.29, 100.83-111.75	0.707
Systolic BP (mmHg), 95% CI	122.53,118.04-127.16	118.90, 114.41-123.39	0.254
Diastolic BP (mmHg), 95% CI	76.25, 71.49-81.37	72.93, 68.27-77.41	0.328
Mean BP (mmHg), 95% CI	92.38, 88.13-96.96	88.85, 84.44-93.12	0.252
Oxygen saturation (%), 95% CI	95.03, 93.75-96.25	96.26, 95.12-97.14	0.125
Average Steady Hb (g/dl), 95% CI	7.73, 7.42-8.05	8.02, 7.72-8.53	0.203
Hb at presentation (g/dl), 95% CI	7.10, 6.72-7.54	7.23, 6.77-7.89	0.678
No Crisis in Preceding 1yr, median	3 (IQR, 1-5)	3 (IQR, 1-4)	0.608
№ of BT in Preceding 1yr, median	0 (IQR, 0-1)	0 (IQR, 0-1)	0.608
Lifetime blood transfusion, median	1 (IQR, 0-2)	1 (IQR, 1-2)	0.608

*** Social class determined using Oyedeji's criteria[126] Hb = Haemoglobin, BT = Blood transfusion BMI = Body Mass Index, BSA = Body Surface Area, SD = Standard Deviation, CI = Confidence Intervals, IQR = Interquartile Range*

Primary Outcome Variable

Effect of arginine supplementation on pulmonary artery pressures and pulmonary vascular resistance

The baseline mean pulmonary artery pressure of patients that received arginine was 26.32 mmHg (95% CI, 23.50-29.14) while in those that had placebo it was 24.44mmHg (95% CI, 21.87-27.01), p = 0.325. Following supplementation, the mean pulmonary pressure reduced to 17.74 mmHg (95% CI, 15.57-19.91) in the arginine arm and 21.75 mmHg (95% CI, 19.58-23.92) in the placebo arm, p = 0.010 (Figure 4.2). Thus, the change in mean pulmonary artery pressure was higher with arginine (8.58 mmHg; 95% CI, 5.08-12.08) than placebo (2.69 mmHg; 95% CI, -0.61-5.99), p = 0.002 (Table 4.2). Overall, arginine therapy was associated with a 21.5% reduction in mean pulmonary artery pressure**.** In addition, there was a significant reduction in the right atrial pressure [25.2% (arginine group) versus 9.7% (placebo group), p < 0.001] and tricuspid regurgitation velocity [30.8% in arginine arm versus 11.1% in placebo arm, p = 0.001] following arginine supplementation.

Similarly, arginine supplementation was associated with a greater reduction in pulmonary vascular resistance than the placebo. Baseline values were 1.46 Wu (95% CI, 1.36-1.56) and 1.37 Wu (95% CI, 1.18-1.56) for arginine and placebo arms respectively, while post-supplementation values were 1.07 Wu (95% CI, 0.85-1.29) for arginine group and 1.25 Wu (95% CI, 1.02-1.48) for placebo group. Therefore, the mean change in pulmonary vascular resistance was higher for patients that received arginine [0.39 Wu (95% CI, 0.17-0.61)] than those that had placebo [0.12 Wu (95% CI, -0.17-0.41)], p= 0.001.

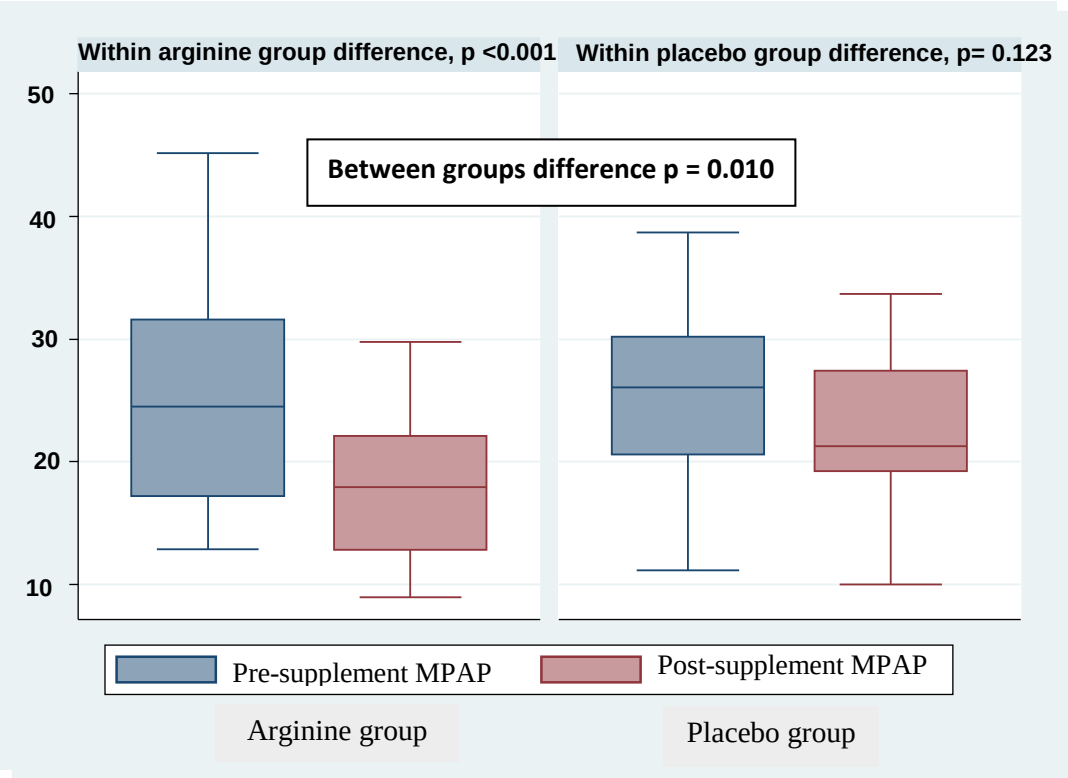


Figure 4.2: Boxplot of mean pulmonary artery pressure (MPAP) by treatment group showing the marked change in the 25th and 75th percentile as well as the median value of the mean pulmonary artery pressure in the arginine and placebo groups

Table 4.2: Impact of arginine supplementation on indices of pulmonary haemodynamics

Pulmonary indices		**Arginine**	**Placebo**	**p-value**
		Mean (95%CI)	**Mean (95%CI)**	
		Pre =35, Post =22	**Pre = 31, Post = 25**	
Right Atrial Pressure (mmHg)	Mean (pre-supplement)	11.29(10.16-12.42)	10.67(9.52-11.82)	0.438
	Mean (post-supplement)	8.44(6.66-10.22)	9.64(8.24-11.04)	0.268
	Change in mean	2.85(0.89-4.81)	1.03(-0.72-2.78)	**<0.001**
	% change in mean	25.2%	9.7%	
Tricuspid regurgitation Velocity (m/s)	Mean (pre-supplement)	2.47(2.30-2.64)	2.25(1.92-2.58)	0.215
	Mean (post-supplement)	1.70(1.33-2.07)	1.99(1.61-2.37)	0.272
	Change in mean	0.76(0.53-0.99)	0.25(0.09-0.41)	**0.001**
	% change in mean	30.8%	11.1%	
Tricuspid regurgitation Gradient (mmHg)	Mean (pre-supplement)	25.09(21.90-28.28)	21.77(17.60-25.94)	0.197
	Mean (post-supplement)	13.42(9.34-17.50)	18.04(13.16-22.92)	0.152
	Change in mean	11.68(8.41-14.95)	3.72(0.37-7.07)	**0.001**
	% change in mean	46.6%	17.1%	
Pulmonary artery systolic pressure (mmHg)	Mean (pre-supplement)	36.65(33.30-40.00)	34.04(29.55-38.53)	0.339
	Mean (post-supplement)	22.33(17.72-26.94)	28.77(23.92-33.61)	0.056
	Change in mean	14.32(10.81-17.83)	5.28(2.56-8.00)	**<0.001**
	% change in mean	39.1%	15.5%	
Pulmonary regurgitation velocity (m/s)	Mean (pre-supplement)	1.37(1.11-1.63)	1.33(1.08-1.58)	0.826
	Mean (post-supplement)	1.17(0.88-1.46)	1.33(1.06-1.60)	0.411
	Change in mean	0.19(-0.07-0.45)	0.01(-0.25-0.27)	0.329
	% change in mean	13.9%	0.8%	
Pulmonary regurgitation gradient (mmHg)	Mean (pre-supplement)	9.15(6.27-12.03)	8.14(5.51-10.77)	0.604
	Mean (post-supplement)	6.82(4.23-9.41)	8.56(5.50-11.62)	0.388
	Change in mean	2.33(-0.43-5.08)	-0.42(-3.61-2.77)	0.186
	% change in mean	25.5%	-5.2%	
Pulmonary Artery Diastolic Pressure (mmHg)	Mean (pre-supplement)	21.15(17.93-24.37)	19.64(17.08-22.02)	0.466
	Mean (post-supplement)	15.44(12.24-18.64)	18.24(15.16-21.32)	0.201
	Change in mean	5.71(2.81-8.61)	1.41(-2.18-5.00)	0.060
	% change in mean	27.0%	7.2%	
Mean PAP (mmHg)	Mean (pre-supplement)	26.32(23.50-29.14)	24.44(21.87-27.01)	0.325
	Mean (post-supplement)	17.74(15.57-19.91)	21.75(19.58-23.92)	**0.010**
	Change in mean	8.58(5.08-12.08)	2.69(-0.61-5.99)	**0.002**
	% change in mean	32.6%	11.1%	
Pulmonary Vascular Resistance (Wu)	Mean (pre-supplement)	1.46 (1.36-1.56)	1.37(1.18-1.56)	0.403
	Mean (post-supplement)	1.07(0.85-1.29)	1.25(1.02-1.48)	0.254
	Change in mean	0.39(0.17-0.61)	0.12(-0.17-0.41)	**0.001**
	% mean change	26.7%	8.8%	

PAP = pulmonary artery pressure, Wu = Wood's Unit

When the indices of pulmonary haemodynamics were stratified according to severity, the prevalence of highly elevated pulmonary artery systolic pressure (PASP >40 mmHg) and mean pulmonary artery pressure (MPAP >30 mmHg) which were similar at baseline became much lower in the arginine group at completion of supplementation, Table 4.3. Arginine supplementation tended to be associated with a greater reduction in the proportion of children having TRV >2.5 m/s (44.1% vs. 20.1%, p = 0.064), PASP >30 mmHg (35.5% vs. 21.2%, p = 0.251), MPAP >25 mmHg (44.1% vs. 18.8%, p = 0.050), even though the difference fell short of statistical significance, Figure 4.3.

Table 4.3: Effect of arginine supplementation on the prevalence of abnormal pulmonary haemodynamics

Indicators of abnormal Pulmonary haemodynamics	**Value**	**Number of patients with abnormal pulmonary haemodynamics**		**P value**	**Number of patients with abnormal pulmonary haemodynamics**		**P value**
		Before arginine N=35 (%)	**After arginine N=22 (%)**		**Before placebo N=31 (%)**	**After placebo N=25 (%)**	
TRV (m/s)	2.5-2.9	18 (51.4)	5 (26.3)	0.127	19 (61.3)	13 (54.2)	0.443
	>3	4 (11.4)	0 (0)		2 (6.5)	0 (0)	
	Total	22 (62.9)	5 (22.7)		21(67.7)	13 (52.0)	
PASP (mmHg)	30-40	11 (31.4)	6 (31.6)	**0.037**	11 (35.5)	8 (33.3)	0.519
	41-50	16 (45.7)	1 (5.3)		14 (45.2)	6 (25.0)	
	Total	27 (77.1)	7 (23.3)		25 (80.6)	14 (56.0)	
MPAP (mmHg)	25-29	6 (17.1)	3 (15.8)	**0.046**	10 (32.5)	6 (25.0)	0.437
	>30	11 (31.4)	0 (0)		9 (29.0)	3 (12.5)	
	Total	17 (48.6)	3 (13.6)		19 (61.3)	9 (36.0)	
PVR (Wu)	>2	3 (8.6)	0 (0)	0.292	3 (9.7)	1 (4.2)	0.626
PCWP (mmHg)	>10	4 (11.4)	4 (21.1)	0.703	4 (12.9)	4 (16.7)	>0.999

NB: *TRV = Tricuspid regurgitation velocity; PASP = Pulmonary artery systolic pressure*
MPAP=Mean pulmonary artery pressure, PVR = Pulmonary vascular resistance
PCWP = Pulmonary wedge capillary pressure

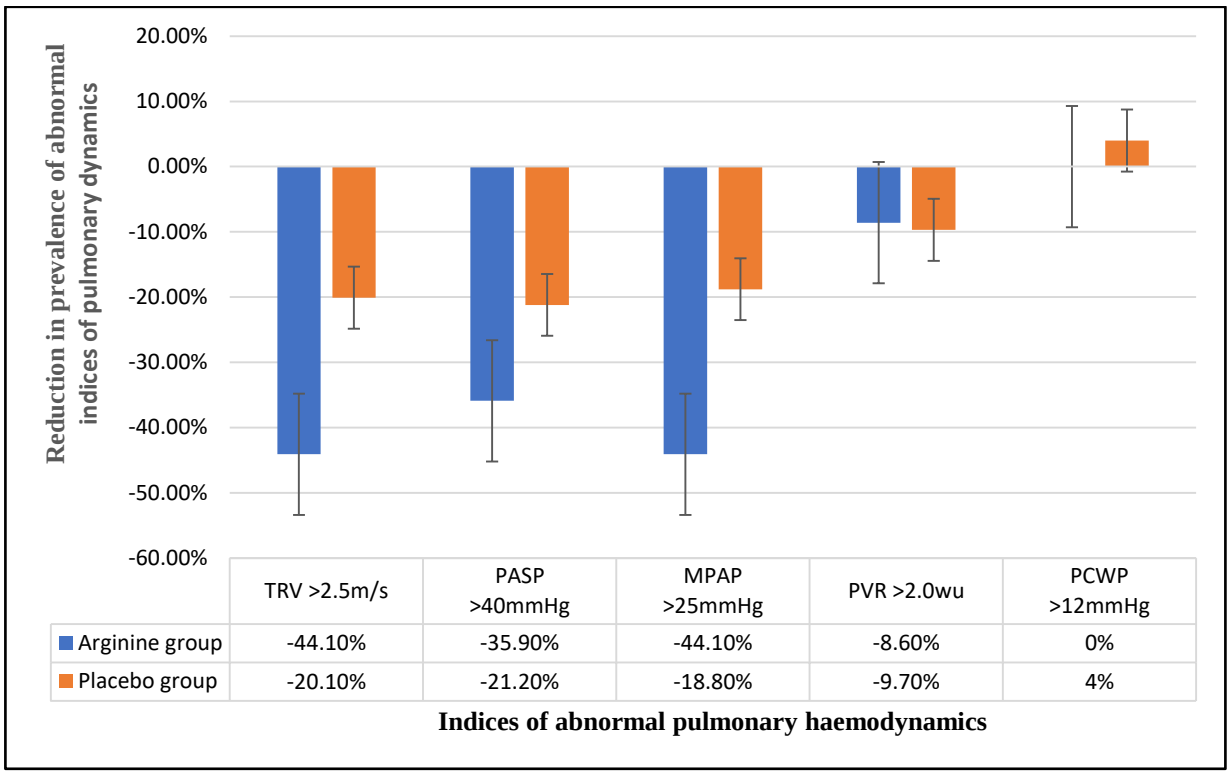


Figure 4.3: Effect of $_{L}$-arginine in reducing the prevalence of abnormal indices of pulmonary haemodynamics
TRV = Tricuspid regurgitation velocity; PASP = Pulmonary artery systolic pressure
MPAP=Mean pulmonary artery pressure, PVR = Pulmonary vascular resistance
PCWP = Pulmonary wedge capillary pressure

Relationship between indices of pulmonary artery pressures and plasma amino acids levels

As depicted in Figure 4.4, patients with high mean pulmonary artery pressure (MPAP >25 mmHg) tended to have lower plasma arginine level [116.86µmol/l (IQR, 93.19-165.99)] compared to those with normal mean pulmonary pressure [165.53 µmol/l (IQR, 118.38-239.39)], suggesting an inverse relationship between plasma arginine level and mean pulmonary artery pressure.

Similarly, patients with high TRV jet (TRV >2.5 m/s) had lower plasma arginine level [129.44 µmol/l (IQR, 99.42-198.11, high TRV jet) vs. 163.19 µmol/l (IQR, 101.93-239.69, low TRV jet)], p = 0.020 and lower percentage increase in plasma arginine: 15.54µmol/l (IQR, -25.75-31.45, high TRV group) vs. 66.1 µmol/l (IQR, 16.4-177.7, low TRV), p = 0.035 and lower global arginine bioavailability: 11.3 (IQR, -49.4-39.29, high TRV jet) vs. 26.9 (IQR, 9.95-98.0,

low TRV), though the difference in global bioavailability just fell short of statistical significance, p = 0.051, Figure 4.5.

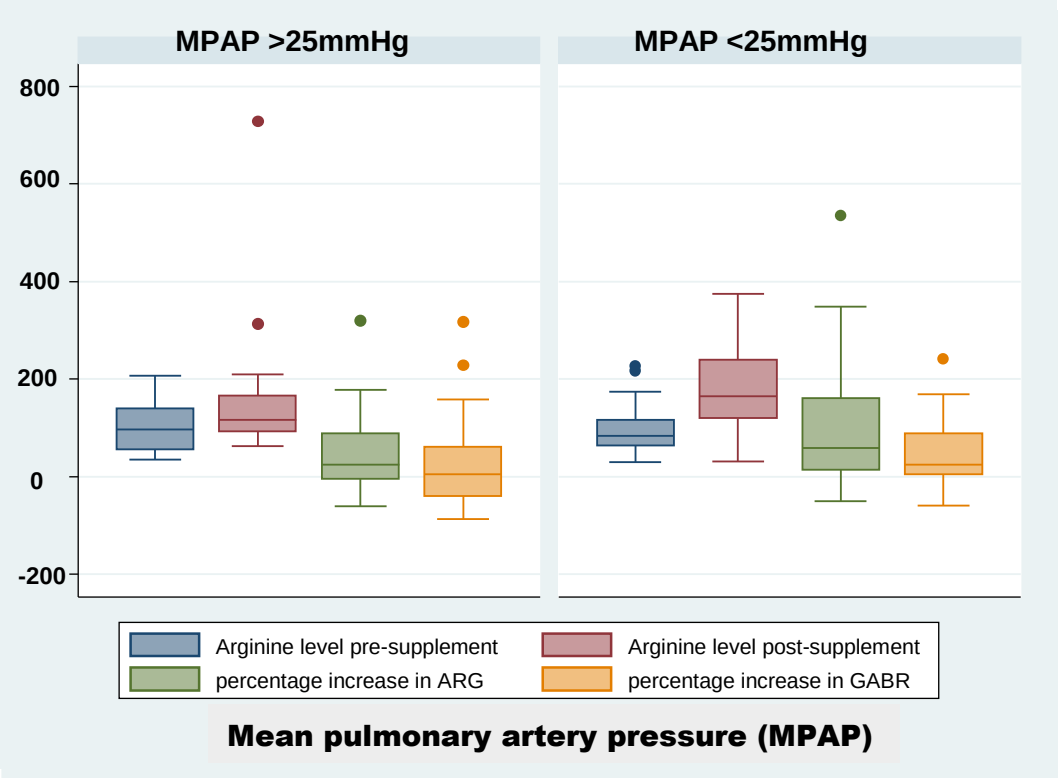


Figure 4.4: Relationship between the mean pulmonary artery pressures and plasma arginine levels pre- and post-arginine supplementation. ARG = Arginine, GABR = Global arginine bioavailability ratio

Consequently, children that received arginine had lower tricuspid regurgitation velocity compared to the placebo group, Figure 4.6. The median TRV in the arginine group was 2.54 m/s (IQR, 1.94-2.84) at baseline but it decreased to 2.07 m/s (IQR, 1.84-2.34) after arginine supplementation. In the placebo group on the other hand, the baseline TRV of 2.61 m/s (IQR, 2.27-2.79) got reduced to 2.46 m/s (1.55-2.60) after supplementation. The difference in response of TRV to arginine supplementation was statistically significant, $p < 0.001$. The myocardial ischaemia score, on the other hand, became much lower after arginine supplementation compared to the placebo where no changes to the ischaemia score were observed, $p<0.001$.

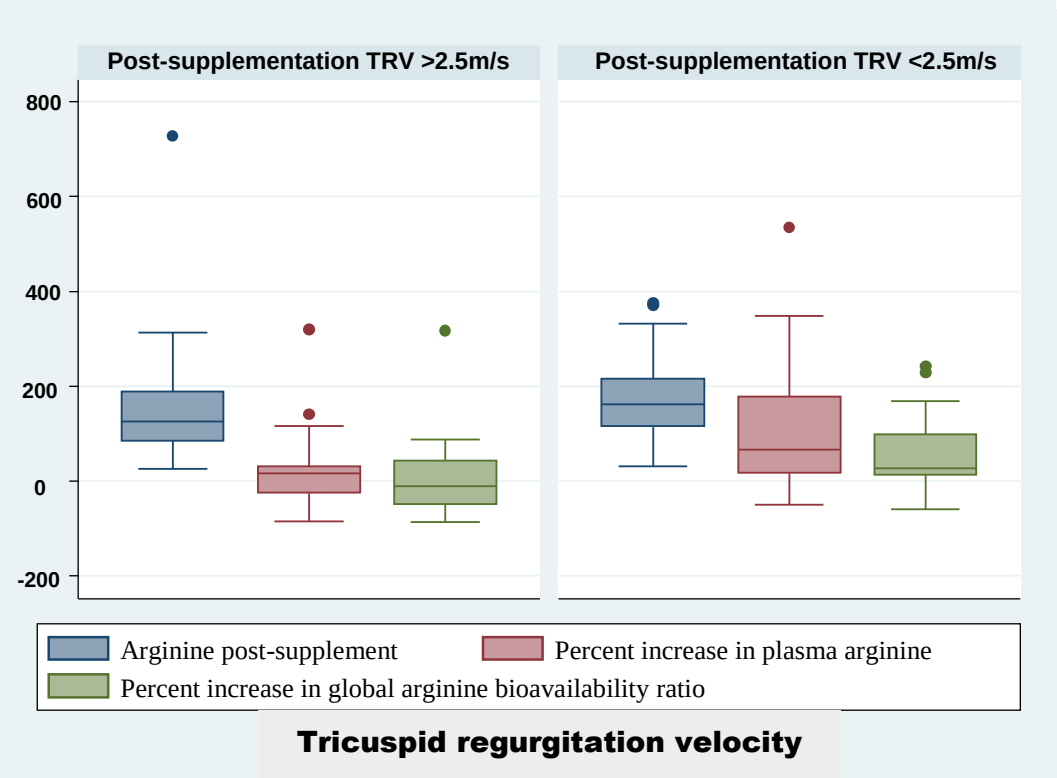


Figure 4.5: Relationship between post-supplement plasma arginine levels, percentage increase in plasma arginine and global arginine bioavailability ratio, and the tricuspid regurgitation jet velocity

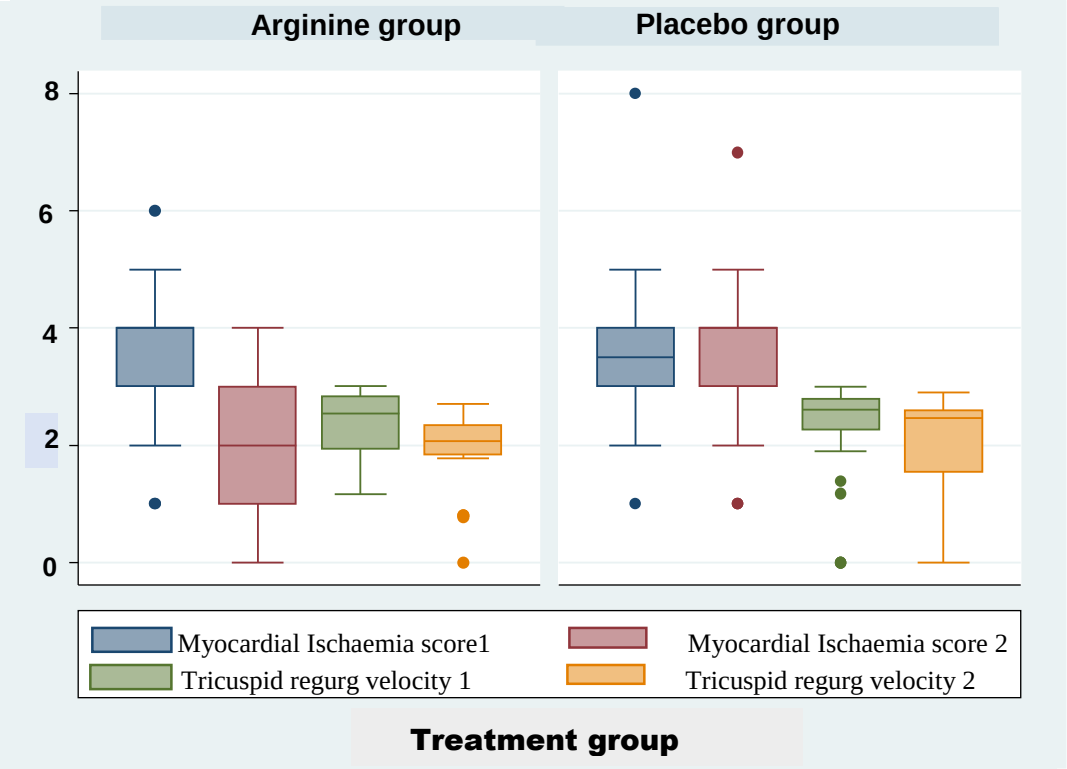


Figure 4.6: Response of myocardial ischaemia scores and tricuspid regurgitation velocity to arginine supplementation, p-value for differences in response is less than 0.001. Regurg = Regurgitation

Effects of $_{L}$-arginine therapy on blood pressure of children with sickle cell crisis

The mean systolic and diastolic BP at baseline were comparable between the two groups and are displayed in Tables 4.4A and B. Mean systolic BP declined from 122.6±13.27 mmHg at day 0 to 109.8±8.75 mmHg at day 5 of arginine therapy, producing a difference in mean of 12.84 ±3.20 mmHg (95% CI, 5.70-19.97), implying an 11% reduction in systolic BP. In the placebo group, only 6.3% reduction was achieved, from a mean of 118.9±12.21 mmHg to 111.35±8.14 mmHg [with a mean difference of 7.55±1.49 mmHg (95% CI, 1.92-13.19)] within the same duration. Thus, arginine supplementation was associated with a between-group difference of 5.29 mmHg (95% CI, 4.04-6.54) in systolic blood pressure, which is statistically significant, p <0.001. Similarly, the mean diastolic BP recorded a 13% reduction in the arginine group (from 76.43±14.38 mmHg to 66.47±7.02 mmHg) as against the 7.5% reduction in the placebo group (from 72.84±12.46 to 67.42±10.05 mmHg). The difference in the mean change between the arginine and placebo group (7.18 mmHg) is statistically significant, p <0.001.

However, the day-to-day effect of arginine on blood pressure in the two groups failed to reach statistical significance.

Table 4.4A: Systolic blood pressure patterns in sickle cell pain crisis following arginine therapy

Day of assessment	Arginine group		Placebo group		P-value
	N	Mean Systolic Blood Pressure (95% CI) (mmHg)	n	Mean Systolic Blood Pressure (95% CI) (mmHg)	
Baseline	35	122.61±3.27 (118.04-127.16)	31	118.90±12.25 (114.41-123.40)	0.089
Day 1	34	119.44±13.45 (114.75-124.13)	31	117.41±11.70 (113.19-121.63)	0.518
Day 2	33	111.91±11.0 (108.00-115.82)	31	111.13±11.16 (107.04-115.22)	0.779
Day 3	31	108.71±13.27 (104.57-112.84)	31	109.48±21.26 (101.69-117.20)	0.865
Day 4	26	108.78±9.06 (105.11-112.43)	28	108.86±8.38 (105.61-112.11)	0.979
Day 5	22	109.76±8.75 (105.27-114.26)	26	111.35±8.14 (108.06-114.63)	0.515
Difference between baseline and day 5 BP		12.84 (95% CI, 5.70-19.97)		7.55 (95% CI, 1.92-13.19)	**<0.001**

Table 4.4B: Diastolic blood pressure patterns in sickle cell pain crisis following arginine therapy

Day of assessment	Arginine group		Placebo group		P-value
	N	Mean Diastolic Blood Pressure (95% CI) (mmHg)	n	Mean Diastolic Blood Pressure (95% CI) (mmHg)	
Baseline	35	76.43±14.38 (71.49-81.37)	31	72.84±12.46 (68.27-77.41)	0.286
Day 1	34	75.79±14.25 (70.82-80.77)	31	73.38±11.61 (69.19-77.56)	0.460
Day 2	33	67.70±10.58 (63.95-71.45)	31	69.55±10.41 (65.73-73.37)	0.484
Day 3	31	65.32±11.40 (61.14-69.50)	31	69.06±11.45 (64.86-73.26)	0.202
Day 4	26	65.19±9.43 (61.58-69.06)	28	68.79±11.21 (64.44-74.06)	0.209
Day 5	22	66.47±7.02 (62.86-70.08)	26	67.42±10.05 (63.36-71.48)	0.626
Difference between baseline and day 5 BP		11.23 (95% CI, 4.75-17.22)		4.05 (95% CI, -2.15-10.26)	**<0.001**

Effects of $_{L}$-arginine therapy on blood pressure percentiles during sickle cell crisis

The proportion of children on arginine with systolic blood pressure (SBP) ≥ 95th percentile on day 1 was 60.0%, but this fell to 8.6% on day 5 (Table 4.5). On the other hand, the proportion of children on placebo with SBP ≥95th percentile declined from 58.1% on day 1 to 32.3% on day 5. The use of arginine is therefore associated with a faster decline in proportion of children with vaso-occlusive crisis-related elevation of systolic blood pressures compared to placebo, p

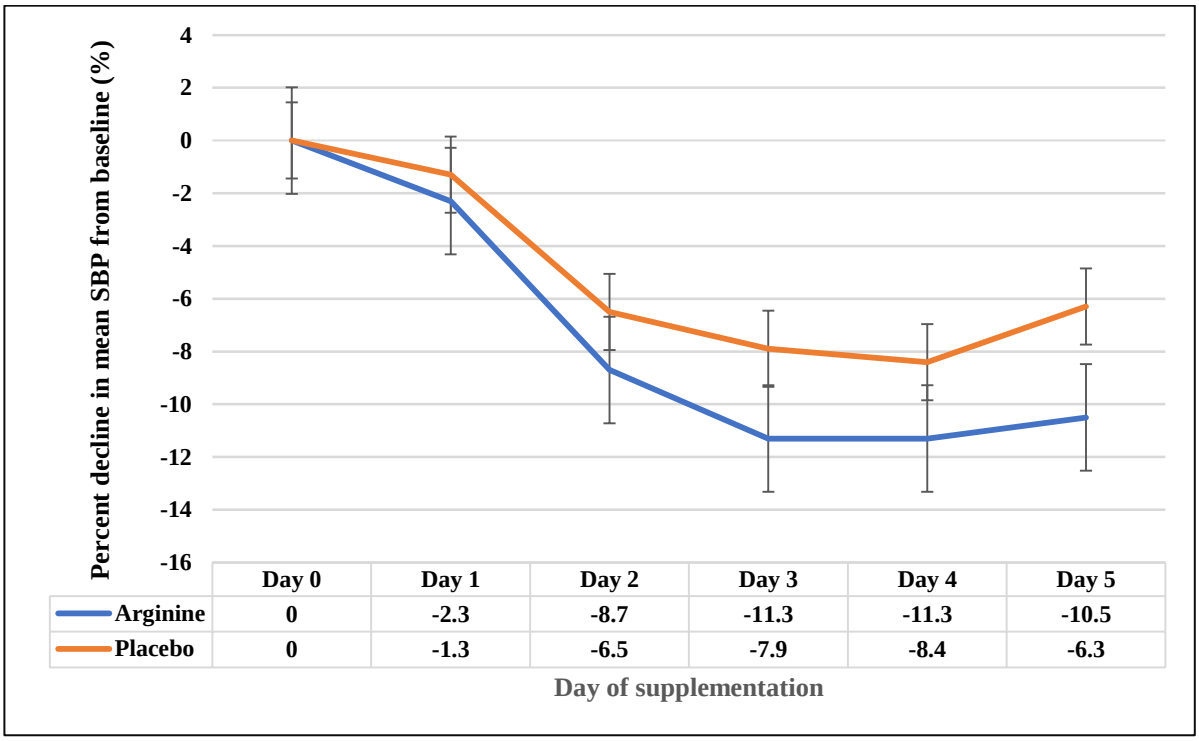
= 0.034 (not shown in table). On a day-to-day analysis, the prevalence of elevated systolic blood pressure was higher in the placebo group particularly from day 3 of the study (p-value of difference in prevalence is 0.028 on day 3 and 0.006 on day 5; Table 4.5).

Diastolic blood pressure (DBP) ≥95th percentile occurred in 42.9% of children on arginine on day 1 but by day 5 only 2.9% of them had DBP ≥95th percentile. For those on placebo, the proportion of children with DBP ≥95th percentile changed from 41.9% on day 1 to 29.0% on day 5. Thus, the proportion of children with significantly elevated diastolic blood pressure was 10 times higher in the placebo group than in the arginine group at day 5 of treatment, p = 0.014. The effect of arginine on the proportion of children with elevated diastolic blood pressure became statistically significant from day 2 (that is after 48 hours of arginine usage), while the proportion of children with elevated systolic blood pressures required at least 72 hours of arginine administration to have a statistically significant reduction.

Table 4.5: Effect of arginine supplementation on the prevalence of elevated blood pressures percentiles (≥90 percentile) based on Intention-to-Treat

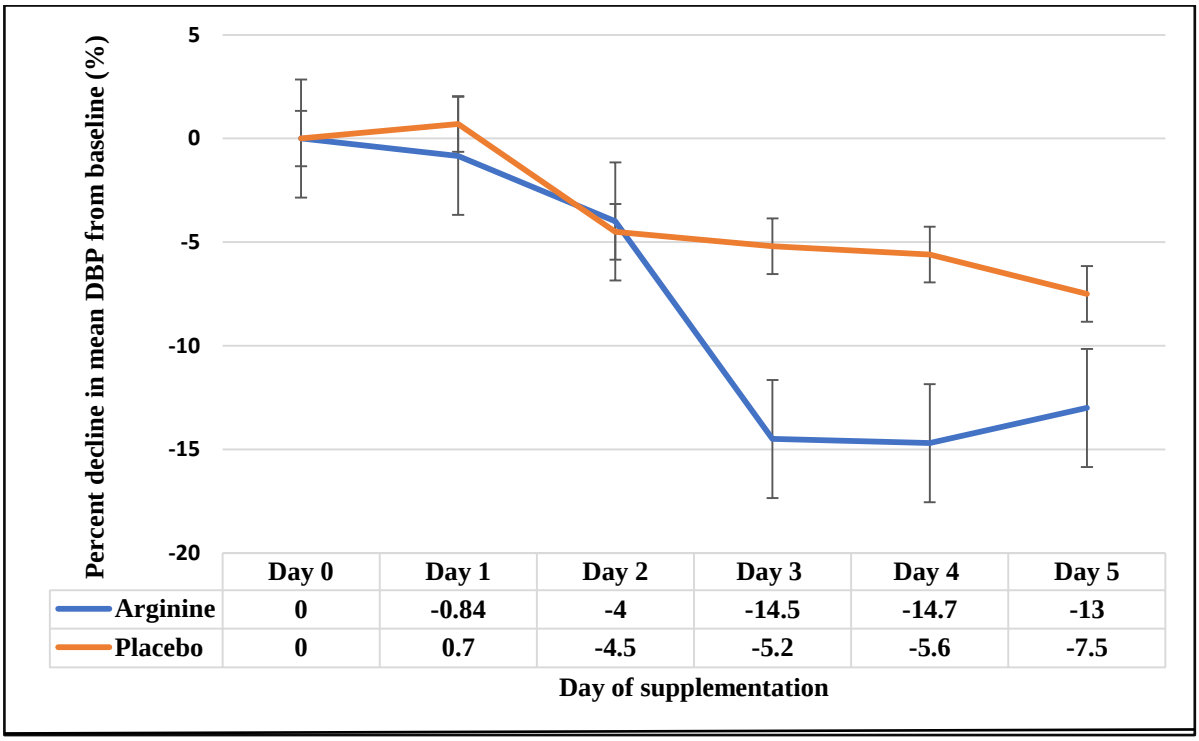
Day of assessment	**Bp Percentiles**	**Systolic Blood Pressure**			**Diastolic Blood Pressure**		
		Arginine (n = 35) (%)	**Placebo (n = 31) (%)**	**p value**	**Arginine (n = 35) (%)**	**Placebo (n = 31) (%)**	**p value**
Day 1	< 90	11(31.4)	12(38.7)		13(37.1)	16(51.6)	
	90- <95	3(8.6)	1(3.2)	0.793	7(20.0)	2(6.5)	0.207
	≥95	21(60.0)	18(58.1)		15(42.9)	13(41.9)	
Day 2	<90	16 (45.7)	11(35.5)		18 (51.4)	19(61.3)	
	90<95	9(25.7)	5(16.1)	0.242	9(25.7)	0(0.0)	**0.010**
	≥95	10(28.6)	15(48.4)		8(22.9)	11(35.5)	
Day 3	< 90	24 (68.6)	12(38.7)		26 (74.3)	17(54.8)	
	90-<95	4(11.4)	8(25.8)	**0.028**	6(17.1)	1(3.2)	**0.005**
	≥95	6(17.1)	11(35.5)		3(8.6)	12(38.7)	
Day 4	<90	28 (80)	17 (54.8)		28 (80.0)	21(67.7)	
	90- <95	2(5.7)	6(19.4)	0.076	5(14.3)	2(6.5)	0.095
	≥95	5(14.3)	8(25.8)		2(5.7)	7(22.6)	
Day 5	< 90	29 (82.9)	14 (45.2)		28 (80.0)	21(67.7)	
	90 - <95	3(8.6)	7(22.6)	**0.006**	6(17.1)	1(3.2)	**0.005**
	≥95	3(8.6)	10(32.3)		1(2.9)	9(29.0)	

A.



	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Arginine	0	-2.3	-8.7	-11.3	-11.3	-10.5
Placebo	0	-1.3	-6.5	-7.9	-8.4	-6.3

B.



	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Arginine	0	-0.84	-4	-14.5	-14.7	-13
Placebo	0	0.7	-4.5	-5.2	-5.6	-7.5

Figure 4.7: Daily percentage decline in the mean value of systolic (upper figure) and diastolic (lower figure) blood pressures from the baseline in the arginine and placebo groups, p value for trend < 0.001. SBP – Systolic blood pressure, DBP – Diastolic blood pressure

Effect of supplementation on ventricular function and wall stress

The mean values of indices of left and right ventricular systolic function were not affected by arginine, Table 4.6. Complete data for computation of left ventricular wall stress were obtained in 61 patients only and these were analysed. Meridional and circumferential wall stress changes during crisis: meridional wall stress decreases while circumferential wall stress increases. Arginine supplementation did not affect the direction of change in the mean values of the wall stress, p = 0.099 for meridional wall stress and 0.129 for circumferential wall stress (Table 4.6).

Table 4.6: Indices of left ventricular function among the study groups

Indices	Pre-Arginine	Post-Arginine	Mean change	Pre-Placebo	Post-Placebo	Mean change	P
LVIDD (cm)	4.59±0.62	4.61±0.54	-0.03±0.41	4.43±056	4.61±0.56	-0.19±0.41	0.559
LVIDS (cm)	2.89±0.48	2.99±0.45	-0.09±0.43	2.78±0.41	2.97±0.46	-0.19±0.36	0.645
EF (%)	66.88±7.79	64.55±6.58	2.33±7.65	67.07±5.67	63.42±7.09	3.65±7.56	0.952
FS (%)	37.27±6.06	35.28±6.76	2.01±5.84	37.55±4.03	34.58±5.21	2.96±5.40	0.512
VCFS (circ/sec)	0.10±0.02	0.10±0.02	0.00±0.02	0.10±0.02	0.09±0.02	0.01±0.02	0.762
CVCFS (circ/sec)	0.08±0.02	0.09±0.07	-0.02±0.07	0.08±0.02	0.10±0.14	-0.02±0.14	0.762
Meridional WS	94.45±31.64	137.57±28.24	-43.12±38.75	86.00±26.71	142.97±25.74	-56.97±20.55	0.099
Circumferential WS	220.03±42.45	131.97±31.08	88.06±58.11	204.93±32.89	138.39±33.40	66.54±48.45	0.129
LVTei index	0.30±0.25	0.34±0.12	-0.05±0.21	0.32±0.12	0.41±0.12	-0.1±0.13	0.257
MAPSE (cm)	1.79±2.04	1.42±0.25	0.37±2.03	1.44±0.25	1.43±0.30	0.02±0.29	0.367
LV Stroke volume	55.45±17.39	54.33±16.91	1.12±10.82	60.68±20.69	59.28±17.91	1.40±9.89	0.918
Cardiac index (L/min/m^2)	4.92±1.17	4.25±1.10	0.67±0.33	5.12±1.42	4.88±1.57	0.24±0.40	**0.037**
TAPSE (cm)	2.18±0.44	2.15±0.39	0.03±0.39	2.21±0.47	2.09±0.37	0.11±0.46	0.452
TAPSE Z	0.057±2.83	0.514±1.86	-0.456±2.59	0.919±2.14	-0.203±2.77	1.121±2.57	0.102
RV Tei Index	0.28±0.09	0.259±0.12	0.02±0.14	0.213±0.08	0.218±0.10	-0.004±0.10	0.603
RVFAC (%)	44.49±10.13	43.08±11.13	1.41±15.52	44.28±9.22	42.48±7.77	1.81±10.30	0.918

LVIDD = Left ventricular internal dimension at end diastole, LVIDS = Left ventricular internal dimension at end systole, EF = Ejection fraction, FS = Fractional shortening, VCFS = Velocity of circumferential fibre shortening, LV = Left ventricle, WS = Wall stress, MAPSE = Mitral annular plane systolic excursion, TAPSE = Tricuspid annular plane systolic excursion, RVFAC = Right ventricular fractional area change

Mean change in indices of diastolic function following supplementation was also not significant except for mitral inflow deceleration time and pulmonary vein S/D ratio that is higher in the arginine group than in the placebo group (Table 4.7).

Table 4.7: Mean change in cardiac indices of diastolic function following supplementation

Cardiac indices of diastolic function	**Arginine group**	**Placebo group**	***P*-value**
	Mean±SD change	**Mean±SD change**	
Mitral inflow E waves (m/s)	0.06±0.15	0.01±0.18	0.281
Mitral inflow A waves (m/s)	0.07±0.32	0.01±0.31	0.471
Mitral inflow E/A	-0.16±0.62	-0.26±0.70	0.570
Tricuspid inflow E/A	0.01±0.26	0.02±0.35	0.899
Mitral E/E´	-0.12±1.50	-0.38±1.10	0.448
Mitral inflow DT (m/s)	11.85±43.26	-25.86±36.33	**0.038**
Pulmonary vein S waves (m/s)	-0.03±0.19	0.03±0.10	0.520
Pulmonary vein D waves (m/s)	0.14±0.23	0.04±0.18	0.151
Pulmonary vein S/D	-0.18±0.23	-0.02±0.21	**0.036**
Pulmonary vein Ar waves (m/s)	0.08±0.15	0.05±0.13	0.459
Pulmonary wedge capillary pressure (mmHg)	-0.14±1.86	-0.47±1.37	0.447

Impact of L-arginine supplementation on the prevalence of ventricular dysfunction in sickle cell vaso-occlusive pain crisis and acute chest syndrome

Table 4.8 shows the prevalence of ventricular dysfunction among the patients studied and the effect of supplement on the dysfunction. Arginine usage tended to reduce the prevalence of ventricular dysfunction: EF <55% occurred in 11.4% of children at presentation but the proportion reduced to 5.3% on day 5 of arginine supplementation, thus achieving a 53.5% reduction in prevalence of low EF. For children on the placebo arm, the proportion of children with EF <55%, increased from 6.5% at presentation to 16.7% on day 5 of placebo. The magnitude of change in the prevalence of LVEF < 55% was statistically significant among the arginine group compared to the change in the placebo group, $p = 0.019$.

The proportion of children with LV Tei index >0.45 decreased by 9.5% in the arginine arm but increased by 18.5% on the placebo arm by day 5 (p = 0.001). An increase in LV end-diastolic and end-systolic dimensions was documented in patients experiencing a crisis. The

administration of arginine was associated with a reduction in the proportion of children with LV dilation by 17.0%, whereas the proportion increased by 8.2% in the placebo group, p = 0.003.

The proportion of children with echocardiographic evidence of left ventricular diastolic dysfunction such as an increase in the mitral E/A ratio tended to increase during crisis, but the extent of increase was less in the arginine group compared to placebo; however, the difference fell short of statistical significance.

Similarly, the proportion of children with echocardiographic evidence of right ventricular dysfunction was less in the arginine group compared to placebo group but the difference failed to reach statistical significance. An exception was the tricuspid E/A ratio <0.8, p = 0.019.

Table 4.8: Prevalence of ventricular dysfunction in children with sickle cell crisis and impact of supplement use on the prevalence of cardiac dysfunction

Indicators of ventricular dysfunction	**Arginine Group**			**Placebo group**			***Z***	***P***
	Pre N=35(%)	**Post N=19(%)**	**Overall % decrease**	**Pre N=31(%)**	**Post N=24(%)**	**Overall % decrease**		
Indices of Left Ventricular systolic dysfunction								
EF < 55%	4 (11.4)	1(5.3)	6.1%	2 (6.5)	4 (16.7)	-10.2%	2.35	**0.019**
LV Tei > 0.45	7 (20.0)	2 (10.5)	9.5%	2 (6.5)	6 (25.0)	-18.5%	3.19	**0.001**
MAPSE Z <-2 SD	6(17.1)	2 (10.5)	6.6%	5 (16.1)	3 (12.5)	3.6%	1.35	0.178
LVIDD Z >2SD	15 (48.6)	6 (31.6)	17%	9 (29.3)	9 (37.5)	-8.2%	3.00	**0.003**
LVIDS z > 2SD	11(31.4)	4 (21.1)	10.3%	6 (19.4)	6 (25.0)	-5.6%	2.32	**0.020**
Indices of Left Ventricular diastolic dysfunction								
Mitral inflow E/A <0.8	1(2.9)	1(5.3)	-2.4%	2 (6.5)	3 (12.5)	-6.0%	0.74	0.461
Mitral inflow E/A >2.5	4 (11.4)	4 (21.1)	-9.7%	2 (6.5)	7 (29.2)	-22.7%	1.45	0.149
Indices of Right Ventricular dysfunction								
RV Tei >0.4	6 (17.1)	1 (5.3)	11.8%	2 (6.5)	1 (4.2)	2.3%	1.48	0.140
TAPSE <-2SD	7(20.0)	0	20.0%	6 (19.4)	0	19.4%	0.06	0.951
Tricuspid inflow E/A <0.8	15 (42.9)	4 (21.1)	21.8%	11 (35.5)	10 (41.7)	-6.2%	2.34	**0.019**

NB:3 patients in the arginine and 1 in the placebo groups had incomplete post-supplement data for Tei index calculation and were therefore excluded from this analysis. LVIDD = Left ventricular internal dimension at end diastole, LVIDS = Left ventricular internal dimension at end systole, EF = Ejection fraction, FS = Fractional shortening, LV = Left ventricle, MAPSE = Mitral annular plane systolic excursion, TAPSE = Tricuspid annular plane systolic excursion

Effects of arginine supplementation on cardiac biomarkers

The median troponin I level at baseline was 0.054 ng/ml (IQR, 0.03-0.40) and 0.050 ng/ml (IQR, 0.01-0.10) for arginine and placebo groups, respectively, but fell to 0.05 ng/ml (IQR, 0.01-0.08) and 0.04 ng/ml (IQR, 0.01-0.11), respectively after supplementation. The Wilcoxon Signed-rank test shows that there is a significant effect of the arginine on troponin I levels (z = 2.364, p = 0.018) whereas the placebo failed to demonstrate the effect, z = -1.097, p = 0.2726, Figure 4.8. Similarly, the median NT-pro BNP levels which were similar at baseline [50.57 pg/ml (IQR, 2-211.5), in the arginine group and 48.59 pg/ml (IQR, 2-197), in the placebo group], reduced to 26.24 pg/ml (IQR, 2-61.14) in the arginine group and 33.0 pg/ml (IQR, 2-52.46) in the placebo group. The Wilcoxon Signed-rank test shows that this effect is significant for arginine (z = 2.296, p = 0.022) but not significant for placebo (z = 1.868, p = 0.062), Figure 4.9.

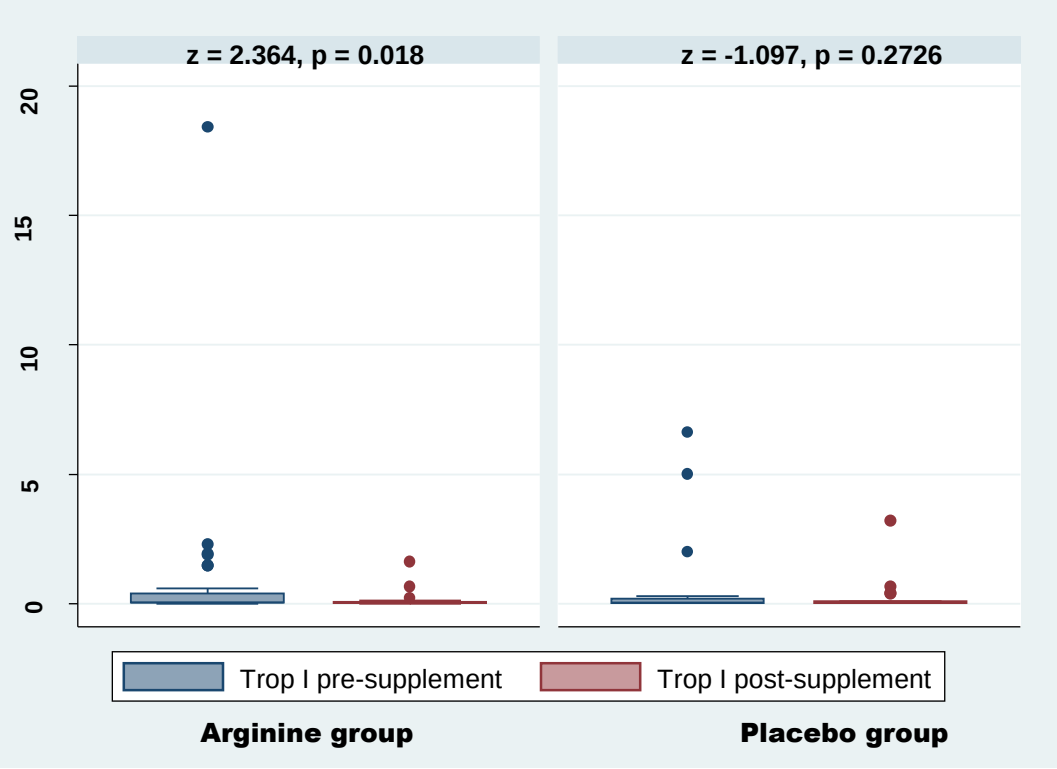


Figure 4.8: Boxplot showing the effect of arginine supplementation on troponin I level. The y-axis shows troponin I levels in ng/ml. Trop I = Troponin I

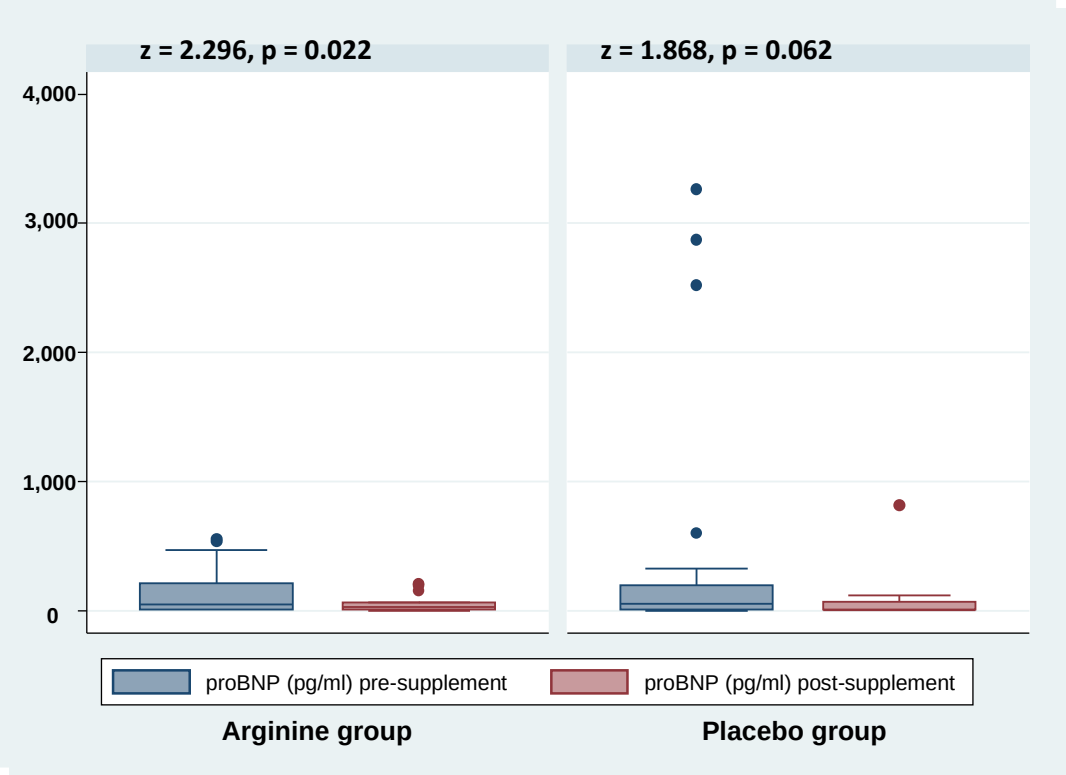


Figure 4.9: Boxplot of response of NT-proBNP to arginine and placebo supplementation, showing a significant difference in the arginine group. NT-proBNP = N-terminal prohormone brain natriuretic peptides

Concomitant medications

Various medications were used concomitantly with the research product. These are drugs routinely used in the management of sickle cell crisis and included non-steroidal anti-inflammatory drugs, opioid analgesics, intravenous fluids, antimalarials and antibiotics. Table 4.9 shows the drugs used in the study patients and the frequency of usage.

Table 4.9: Display of concomitant medications used during sickle cell crisis management

Medications	Arginine group N = 35 (%)	Placebo group N = 31 (%)	χ2	P
Acetaminophen	12 (34.3)	8 (25.8)	0.30	0.582
Artesunate	18 (51.4)	19 (61.3)	0.18	0.669
Coartem	23 (65.7)	21 (67.4)	0.01	0.938
Ceftriaxone	24 (68.6)	31 (100.0)	1.06	0.303
Diclofenac	31 (88.6)	22 (71.0)	0.35	0.551
Dihydrocodeine	20 (57.1)	11 (35.4)	1.13	0.287
Gentamicin	3 (8.6)	9 (29.0)	FET	0.116
Ibuprofen	11 (31.4)	6 (19.4)	0.75	0.388
Intravenous fluids	26 (74.3)	15 (48.4)	1.01	0.291
Ketorolac	18 (51.4)	12 (38.7)	0.41	0.524
Morphine	13 (37.1)	9 (29.0)	0.24	0.621
Pentazocine	16 (45.7)	14 (45.2)	0.01	0.979
Others	30* (85.7)	15 $^{\beta}$ (48.4)	2.05	0.152

**Ciprofloxacin (6), Cefuroxime (3), Diazepam (1), Fansidar (2), P-Alaxin (3), Arteether (3), Azithromycin (3)*
β Ciprofloxacin (2), Clindamicin (3), Arteether (1), Azithromycin (5), FET = Fisher's exact test

Occurrence of adverse events

Occurrence of adverse events was similar in the arginine and placebo groups, p = 0.55 (Figure 4.10). The commonest side effect was vomiting, particularly amongst the arginine group. A significant drop in packed cell volume warranting blood transfusion occurred in 13.9% of patients on arginine and in 15.6% of those on placebo. A fever was recorded in 13.9% and 18.8% of patients on arginine and placebo, respectively. Other side effects documented are shown in Table 4.9. Serious adverse events were not recorded in the arginine group, however,

two patients on placebo developed empyema thoraces warranting closed chest tube thoracotomy.

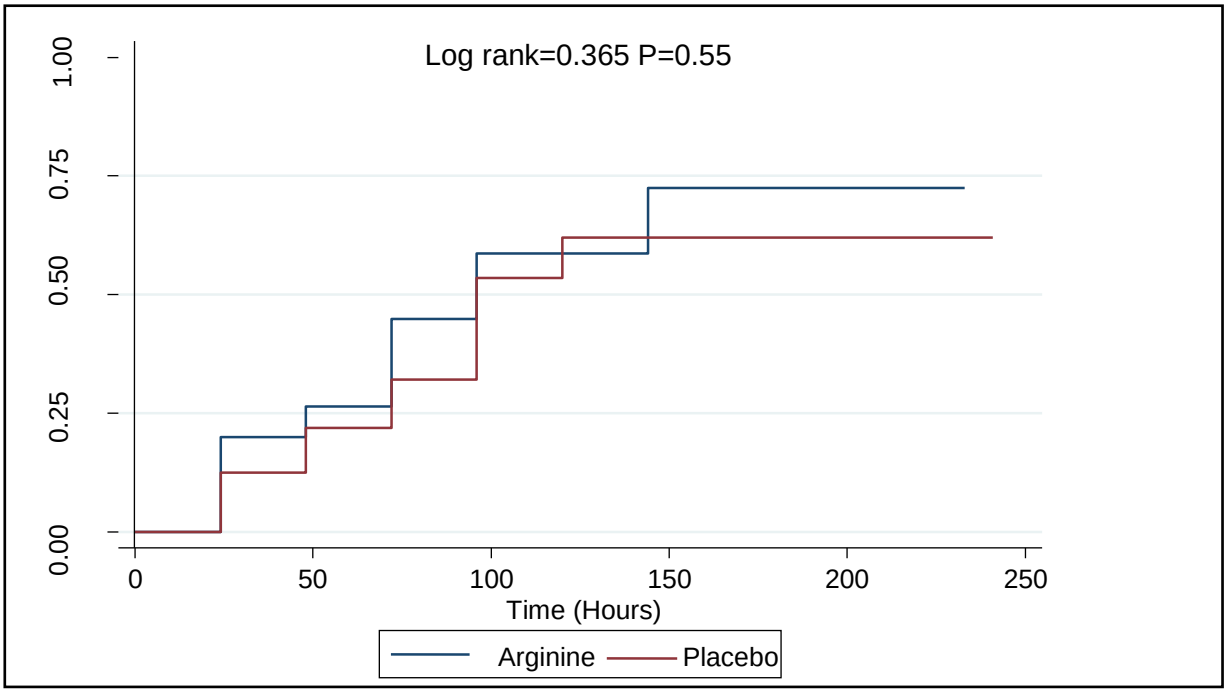


Figure 4.10: Cumulative incidence of adverse events in the study population. The y-axis shows the probability of recording adverse events which tended to increase with time

Table 4.9: Profile of adverse events reported during the supplementation period

Adverse Events	**Arginine (n=35) (%)**	**Placebo (n=31) (%)**	**Test statistic**	***P*-value**
Vomiting	7 (20.0)	1(3.2)	FET	0.127
Nausea	1 (2.9)	0	FET	>0.999
Diarrhoea	1(2.9)	0	FET	>0.999
Fever	5(14.3)	6(19.4)	FET	0.750
Significant fall in PCV	5(14.3)	5(16.1)	FET	>0.999
Abdominal pain	2(5.7)	4(12.9)	FET	0.423
Headache	1(2.9)	2(6.5)	FET	0.603
Rashes (+bullous eruption)	1(2.9)	3(9.7)	FET	0.350
Restlessness	1 (2.9)	0	FET	>0.999
Hiccups	1 (2.9)	0	FET	>0.999
Cough	0	1 (3.2)	FET	0.478
Serious adverse events				
Empyema thoracis	0	2 (6.5)	FET	0.232

4.2 DISCUSSION

Cardiovascular changes in sickle cell crisis are numerous, though often self-limiting, and could alter the clinical course of the disease, therefore, anticipatory care and/or preventive measures are of utmost importance.

Pulmonary hypertension is poorly tolerated by adults with sickle cell crisis.[112] In this study, pulmonary hypertension prevalence among patients with severe VOC was 54.5%. This prevalence is much higher than the 14.1% recorded in patients recruited in steady state[143] but similar to the report of Machado *et al*[62] who demonstrated an increase both in the prevalence and severity of pulmonary hypertension during periods of acute stress such as crisis and exercise. Although the exact cause of elevation in pulmonary pressure remains unknown, various factors have been implicated such as an increase in cardiac output, left ventricular volume overload and nitric oxide-related vasculopathy.[88, 101-103,147]

In the present study, arginine supplementation was associated with a 21.5% reduction in mean pulmonary artery pressure. In an anecdotal study involving 10 adult patients, Mehta *et al*[102] recorded a lower 15.8% reduction in pulmonary artery pressure following intravenous infusion of arginine. Additional evidence that arginine supplementation is associated with a drop in pulmonary artery pressures was provided by Morris *et al*,[35] who showed a much lower (15.2%) reduction in mean pulmonary artery pressure following a 5-day course of oral arginine. Although the magnitude of decline appears small when considering the benefit of arginine in the entire paediatric population, it may be useful in a subset of sickle cell disease patients who are not able to tolerate even small acute increases in pulmonary pressure.

Arginine has also been shown to reduce pulmonary vascular resistance. In the present study, arginine was associated with a 0.27Wu reduction in the pulmonary vascular resistance, which corresponds to 21.6% decline. This is corroborated by the results of Nagaya *et al*[101] and Mehta *et al*[102] which showed a 27.6% decline in pulmonary vascular resistance following arginine administration. The exact mechanism underlying the reduction in pulmonary artery pressure and vascular resistance is not known but may be related to the relaxation of vascular smooth muscle induced by nitric oxide.

Blood pressure (BP) measurements in children with sickle cell vaso-occlusive crisis have shown various degrees of hypertension,[143] including stage 1 and stage 2 hypertension. Some studies in adults have reported that arginine supplementation lowers blood pressure.[148,149] In the present study, arginine supplementation lowered the systolic blood pressure by 5.29 mmHg (95% CI, 4.04-6.54) and the diastolic pressure by 4.54 mmHg (95% CI, 2.98-6.10). This finding is similar to the report of Dong *et al*[150] who, in a meta-analysis of 11 randomized double-blind, placebo-controlled trials involving 387 participants, showed that arginine supplementation significantly lowers the systolic blood pressure by 5.39 mmHg (95% CI, 2.25-8.54, $p = 0.001$) and diastolic blood pressure by 2.66 mmHg (95% CI, 1.54-3.77, $p<0.001$).

The result of the present study is further corroborated by the report of Pezza *et al*[149] who were able to achieve hypertension control by addition of oral arginine to conventional antihypertensive therapy after 3 months of failure to achieve blood pressure stabilization with antihypertensive medication. On the contrary, Schlaich *et al*[151] did not find any effect of arginine on the blood pressure of hypertensive and normotensive adults. The conflicting results of studies on the BP-lowering effects of arginine show the need for further studies.

The antihypertensive mechanism of arginine is not established. Some authors,[152-156] however have suggested a link with nitric oxide-mediated vasodilatation of the renal arteries,[152,154] which causes a decrease in renal vascular resistance and subsequent inhibition of the angiotensin converting enzyme,[155] and hence the normalization of blood pressure.

Ventricular dysfunction (systolic and diastolic dysfunction) depicted by a derangement in the Tei Index, occurred in 20.0% of children with sickle cell crisis in the present study and could be associated with an unfavourable outcome as revealed by a study among adult SCD patients with heart failure in the United States of America, where 2.86% died during admission.[157] Although no death was recorded in the current study, presentation with heart failure implies a poor outcome without appropriate intervention in a child manifesting with a sickle cell crisis.

Arginine supplementation was associated with a 9.5% decrease in prevalence of left ventricular dysfunction (LV Tei Index >0.45) in the present whereas an increase in prevalence was present in the placebo group, suggesting a role for arginine in limiting the negative effects of sickle cell crisis on ventricular function. The right ventricular function was similarly affected by arginine, but the degree of improvement failed to reach statistical significance.

There is overall, a paucity of data on the effect of arginine on ventricular function. One exceptional publication by Bocchi *et al*,[156] demonstrated an increase in stroke volume and cardiac output, and a decrease in heart rate after intravenous $_{L}$-arginine infusion in 7 adults with severe chronic heart failure referred for heart transplant. Other authors, Koifman *et al*,[158] also showed a decrease in blood pressure and peripheral vascular resistance, and an increase in LV ejection fraction following an hour long continuous infusion of arginine in 12 adults with moderate congestive heart failure. The present study confirms a potential role for arginine supplementation in improving cardiac function in patients with sickle cell crisis and warrants further investigation.

Arginine supplementation in this study was also associated with a significant reduction in NT-proBNP and troponin I biomarkers associated with congestive heart failure and myocardial ischaemia and confirms the findings of previous authors.[156] The significant difference in mean between the baseline and post-supplement values suggests that the cardiac dysfunction in sickle cell crisis is LV distension- and ischaemia-related.

The mechanism underlying the improvement in cardiac function with arginine therapy is largely unknown. The effect of arginine could be mediated through different pathways including inhibition of angiotensin converting enzyme,[155] reductions in plasma endothelin I levels,[159] increases in nitric oxide production[160] and enhancement of mitochondrial functions.[161] These mechanisms acting individually or addictively could explain the decrease in systemic vascular resistance, increase in ejection fraction and decrease in heart rate documented in the present study.

In conclusion, oral arginine supplementation is beneficial in ameliorating adverse cardiovascular changes occurring during painful crisis, in particular the control of systemic hypertension, pulmonary hypertension and an improvement in left ventricular function.

4.3. SUMMARY OF MAJOR FINDINGS

Arginine therapy was associated with significant reduction in tricuspid regurgitation velocity, pulmonary artery systolic pressure and mean pulmonary artery pressure. The prevalence of elevated arterial blood pressure was much lower in the arginine group than in the placebo group after arginine supplementation.

The myocardial ischaemia score was much lower with arginine supplementation while the placebo was not associated with changes to the ischaemia score. Arginine usage reduces the prevalence of ventricular dysfunction and lowers the systemic vascular resistance and cardiac biomarker levels.

CHAPTER 5

ARGININE THERAPY AS A NOVEL ADJUVANT IN THE MANAGEMENT OF VASO-OCCLUSIVE PAIN CRISIS

This chapter describes and discusses the results of the investigation into the role of arginine as a novel adjuvant therapy in the management of vaso-occlusive pain crisis.

A part of the findings in this section was presented at the American Society of Hematology meeting and was awarded the ***Best of ASH*** status. The abstract of the presentation was published in the Blood journal as:

Onalo R, Cooper P, Cilliers A, Nnebe-Agumadu U, Oniyangi OO, Oladimeji D, Morris CR. Oral arginine therapy as a novel adjuvant in the management of acute pain in children with sickle cell anaemia in Nigeria: a randomized placebo-controlled trial. Blood 2019; **134** (Suppl_1): 613 doi.org/101182/blood_2019-122510.

The abstract is also published in the Medscape at https://www.medscape.com.

The work has also been accepted for publication in the American Journal of Hematology as:

1. **Richard Onalo**, Peter Cooper, Antoinette Cilliers, Barend C Vorster, Nnebe-Agumadu Uche, Oniyangi O Oluseyi, Victoria D. Onalo, Yunusa Zubairu, Alice U Ayodele-Kehinde, Oladimeji M. Damilare, Janet Figueroa, Claudia R. Morris. Randomized Control Trial of Oral Arginine Therapy for Children with Sickle Cell Anemia Hospitalized for Pain in Nigeria. *American Journal of Hematology* 2020. doi: 10.1002/ajh.26028. IF 5.303 (2018)

5.1 RESULTS

Enrolment

The enrolment of eligible patients followed the pattern depicted in the CONSORT flow diagram in Figure 5.1. A total of 459 sickle cell anaemia patients were hospitalised within the study period, 69 patients were enrolled but one parent withdrew their child (would not allow venepuncture) before randomisation. Many of the patients that participated in the study on the impact of arginine on cardiovascular changes in sickle cell crisis were involved in this study while some other new patients were randomised and added to arrive at a total of 68 patients.

All the study participants received their medications according to the study protocol until discharge or completion of the 15 maximum doses of arginine supplement or placebo. Patients achieving resolution of crisis were discharged home as soon as possible and discontinued from the study. Forty-one patients (60.3%) received the maximum 15 doses of arginine during the admission. All the patients that discontinued the drugs did so because they were discharged home, except for one patient that died on the second day after commencement of therapy.

Baseline characteristics of patients

The 68 randomised patients were between the ages of 5 and 17 years, had a mean age of 10.63 ± 0.41 years and a sex ratio (M:F) of 1.27:1. The mean weight ± SD and height ± SD were 30.97±1.40 kg and 140.75±1.99 cm, respectively, Table 5.1. Twenty-seven (39.7%) were from families of upper socio-economic status (SES) class, 19 (27.9%) were from a middle SES class while 23 (33.8%) came from lower a social class based on the Oyedeji's socio-economic class.[112] Forty-six (67.6%) had a pure vaso-occlusive painful crisis, 17 (25.0%) had mixed crisis (two or more forms of crisis). Seven of the patients with multiple crises experienced a vaso-occlusive painful crisis combined with an acute chest syndrome, another seven had a vaso-occlusive painful crisis together with a haemolytic crisis, one patient had a vaso-occlusive painful crisis with an acute cerebral syndrome (stroke), and the remaining two patients had a vaso-occlusive painful crisis associated with a hepatopathy and a mesenteric infarction. Details of patients' characteristics are displayed in Table 5.1 which shows comparable features between the arginine and placebo groups.

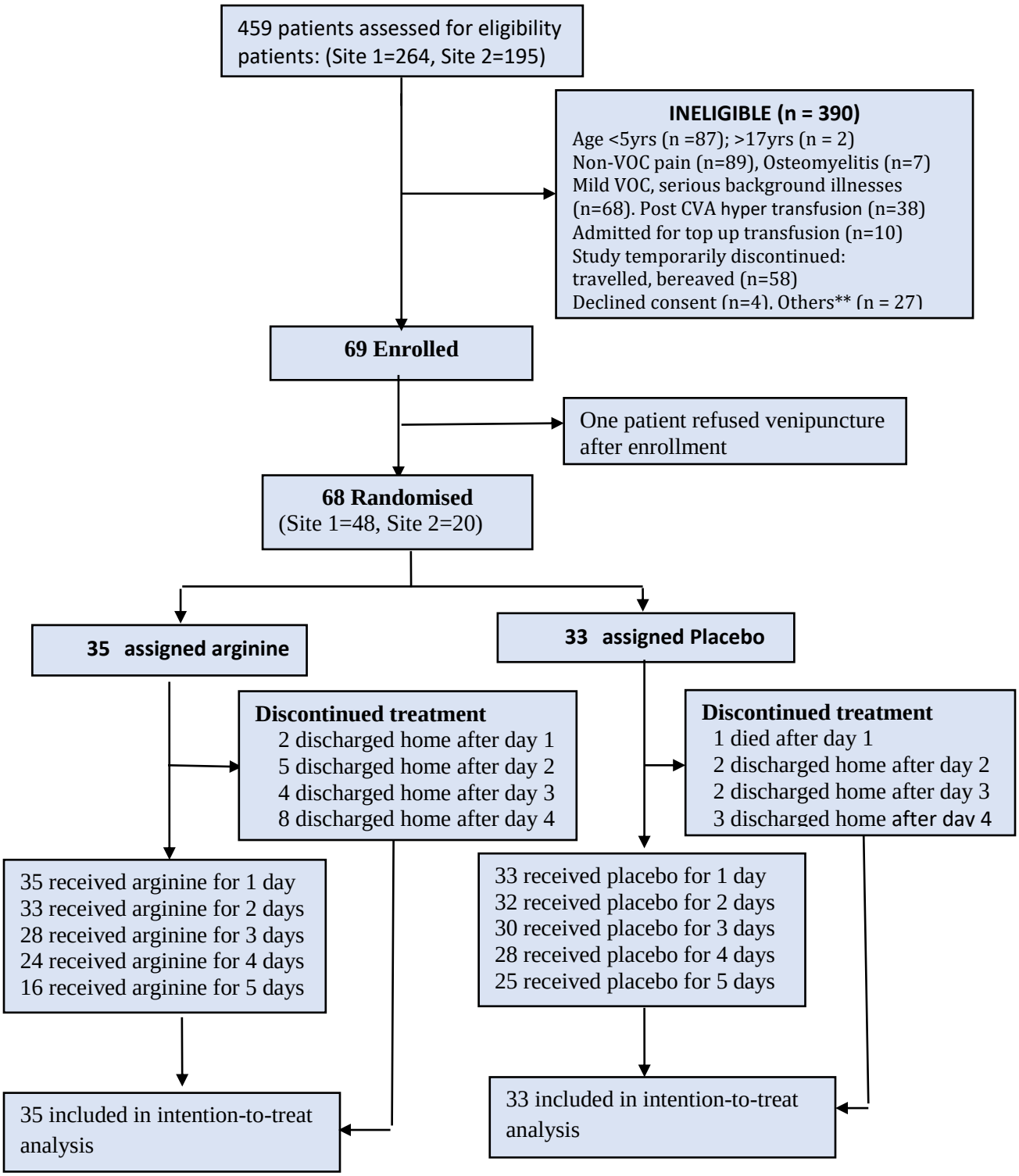


Figure 5.1: CONSORT flow diagram for enrolment and analysis of participants of the arginine as adjuvant for management study.

**Other reasons for the exclusion of eligible patients included guardian not available to give consent (n = 3), patient needed immediate blood transfusion (n = 6), readmission (n= 7), hepatitis (n = 5), treatment commenced before diagnosis of sickle cell anaemia was made (n = 5) and renal failure (n = 1)

Table 5.1: Baseline characteristics in the Intention-to-Treat Population

Parameters	Arginine group (n = 35)	Placebo group (n = 33)	P-value	95% CI
Age (years)	10.65±3.22	10.50±3.54	0.8554	9.82-11.44
Gender				
Male	18 (51.4%)	20 (60.6%)	0.748	-
Female	17 (48.6%)	13 (39.4%)		
Social class				
Upper class	16 (45.7%)	11 (33.3%)	0.536	-
Middle class	10 (28.6%)	8 (24.2%)		
Lower class	9 (25.7%)	14 (42.4%)		
Weight Z score	-1.74±1.93	-1.58±1.84	0.7284	-0.76 to 1.08
Height Z score	-1.09±1.75	-0.64±1.72	0.2899	-0.39 to 1.29
Body Mass Index Z score	-1.37±1.77	-2.23±2.89	0.1389	-2.0 to 0.29
Body Surface Area	1.08±0.20	1.10±0.30	0.7449	-0.1 to 0.1
No of crisis in preceding year$^{\beta}$				
<3	9 (28.1%)	15 (48.4%)	0.254	-
≥3	23 (71.9%)	16 (51.6%)		
Hospitalisation in previous year$^{\beta}$				
<3	25 (78.1%)	27 (87.1%)	0.644	-
≥3	7 (21.9%)	4 (12.9%)		
Blood transfusion previous year$^{\beta}$				
0	18 (56.3%)	15 (48.4%)	0.823	
1-2	14 (43.8%)	16 (51.6%)		
Average steady state Hb$^{\beta}$	7.7±0.9	8.1±1.1	0.1047	7.6 to 8.1
Hb concentration at admission	7.4±1.0	7.1±1.4	0.3108	7.0 to 7.6
Red blood cell count, x10^{12}/L	2.70 (2.22-3.15)*	2.70(2.33-3.30)*	0.4441	2.27 to 4.69
White blood cell count, x10^{6}/L	18.2 (14.7-21.4)*	17.8(14.4-22.9)*	0.9139	17.17 to 30.82
Platelet count, x 10^{6}/L	425 (329-551)*	320 (208-434)*	0.106	326.4 to 407.4
Oxygen saturation (%)	95.1±0.3.7	98.3±2.6	0.129	94.9 to 96.5
Patients with confirmed malaria	6 (17.1%)	6 (18.2%)	0.925	-
Hydroxyurea use				
Yes	5 (14.3%)	5 (12.9%)	0.995	-
No	30 (85.7%)	28 (90.3%)		
Types of crisis				
Vaso-occlusive painful crisis	24 (68.6%)	22 (66.7%)	0.962	-
Acute chest syndrome	2 (5.7%)	3 (9.1%)		
Mixed crisis	9 (25.7%)	8 (24.2%)		
Baseline pain scores	8.72±1.13	8.42±1.26	0.305	
Number of pain sites				
1	6 (17.1%)	9 (27.3%)	0.184	
2-3	24 (68.6%)	14 (42.4%)		
≥4	5 (14.3%)	10 (30.3%)		

***** *reported as median (Interquartile Range); β obtained in 32 patients on arginine and 31 patients on placebo. Social status was classified by Oyedeji method[126] based on the occupation and level of education*

Clinical markers of sickle cell disease severity such as number of crisis, hospital admissions and blood transfusions in the preceding year as well as the average steady state haemoglobin concentration and lifetime blood transfusion rate in the arginine arm were similar to those of patients in the placebo arm (Table 5.1).

Characteristics of pain in the study population

The intensity of pain was even distributed across the various age groups. As shown in Table 5.2, age is not associated with the intensity of pain ($p = 0.926$). The number of pain sites reported ranged from 1 to 13, with a mode of 2. The chest (sternum 23.5% and ribs 11.8%), thigh (20.6%), shin (17.7%), ankle (17.7%), upper arm (17.7%), knee (16.2%) and lower back (16.2%) were the common sites of pain. Other sites included the hip (13.2%), abdomen (13.2%), elbow (11.8%), shoulder (11.8%) and upper back (11.8%). Various parts of the upper and lower limbs were affected with fairly uniform occurrence rates across the age groups. However, ankle and shin pain was more common in children aged 5-8 years, while the sternum, thigh and knee was affected predominantly in children aged 9 years and above. Lumbar vertebrae pain was common in the older age group of children between 9 and 12 years as well as those older than 12 years ($p = 0.098$). Similarly, involvement of the shoulder and wrist were only seen in the older age groups (≥9 years).

Table 5.2: Characteristics of pain in patients studied according to their age groups.

Pain characteristics	5-8 years n=21 (%)	9-12 years n=25 (%)	≥13 years n=22 (%)	Total N = 68 (%)	P value
Intensity of pain					
7	7 (33.3)	7 (28.0)	5 (22.7)	19 (27.9)	
8	3 (14.3)	4 (16.0)	6 (27.3)	13 (19.1)	0.926
9	4 (19.1)	7 (28.0)	4 (18.2)	15 (22.1)	
10	7 (33.3)	7 (28.0)	7 (31.8)	21 (30.9)	
Number of pain sites					
1	3 (14.3)	8 (32.0)	4 (18.2)	15 (22.1)	
2 – 3	15 (71.4)	10 (40.0)	13 (59.1)	38 (55.9)	0.167
4 – 5	1 (4.8)	4 (16.0)	3 (13.6)	8 (11.8)	
≥6	2 (9.5)	3 (12.0)	2 (9.1)	7 (10.3)	
Location of pain					
Upper arm (humerus)	5 (23.8)	4 (16.0)	3 (13.6)	12 (17.7)	0.633
Elbow	5 (23.8)	2 (8.0)	1 (4.5)	8 (11.8)	0.710
Wrist	-	3 (12.0)	1 (4.5)	4 (5.9)	0.588
Hip	1 (4.8)	5 (20.0)	3 (13.6)	9 (13.2)	0.141
Thigh (femur)	2 (9.5)	6 (24.0)	6 (27.3)	14 (20.6)	0.765
Knee	1 (4.8)	6 (24.0)	4 (18.2)	11 (16.2)	0.438
Shin (tibia/fibula)	6 (28.6)	4 (16.0)	2 (9.1)	12 (17.7)	0.851
Ankle	7 (33.3)	2 (8.0)	3 (13.6)	12 (17.7)	0.279
Shoulder	-	3 (12.0)	4 (18.2)	7 (10.3)	0.868
Upper back (thoracic v)*	3 (14.3)	2 (8.0)	3 (13.6)	8 (11.8)	0.292
Lower back (lumbar v)	-	6 (24.0)	5 (22.7)	11 (16.2)	0.098
Sternum	4 (19.1)	7 (28.0)	5 (22.7)	16 (23.5)	0.754
Ribs	2 (9.5)	2 (8.0)	4 (18.2)	8 (11.8)	0.470
Abdomen	4 (19.1)	4 (16.0)	1 (4.5)	9 (13.2)	0.753

**V refers to vertebrae*

Relationship between number of pain sites and intensity of pain

Table 5.3 demonstrates the relationship between number of pain sites in the patients and the intensity of pain reported. There is a strong positive correlation between the intensity of pain and number of pain sites reported on each day of assessment ($p < 0.001$).

Table 5.3: Correlation between number of pain sites and intensity of pain

Day of assessment	R	p-value
Day 1	0.4437	**<0.001**
Day 2	0.5316	**<0.001**
Day 3	0.6243	**<0.001**
Day 4	0.6973	**<0.001**
Day 5	0.7248	**<0.001**

End points

Primary Outcome

Although, the sample size was calculated based on intravenous opioid usage from the Morris 2013 study, the Analgesic Medication Quantification Scale score (MQS) encompasses both opioid and non-opioid analgesics and change in MQS was therefore chosen as the primary outcome measure. To this day, there is no "ideal outcome measure" for sickle cell acute pain studies, as all suggested measures have their limitations.

Pearson correlation analysis of the study showed a good positive correlation between opioid use and MQS as depicted in Figure 5.2. Since opioid use is part of the MQS, MQS represents the best surrogate that could be identified.

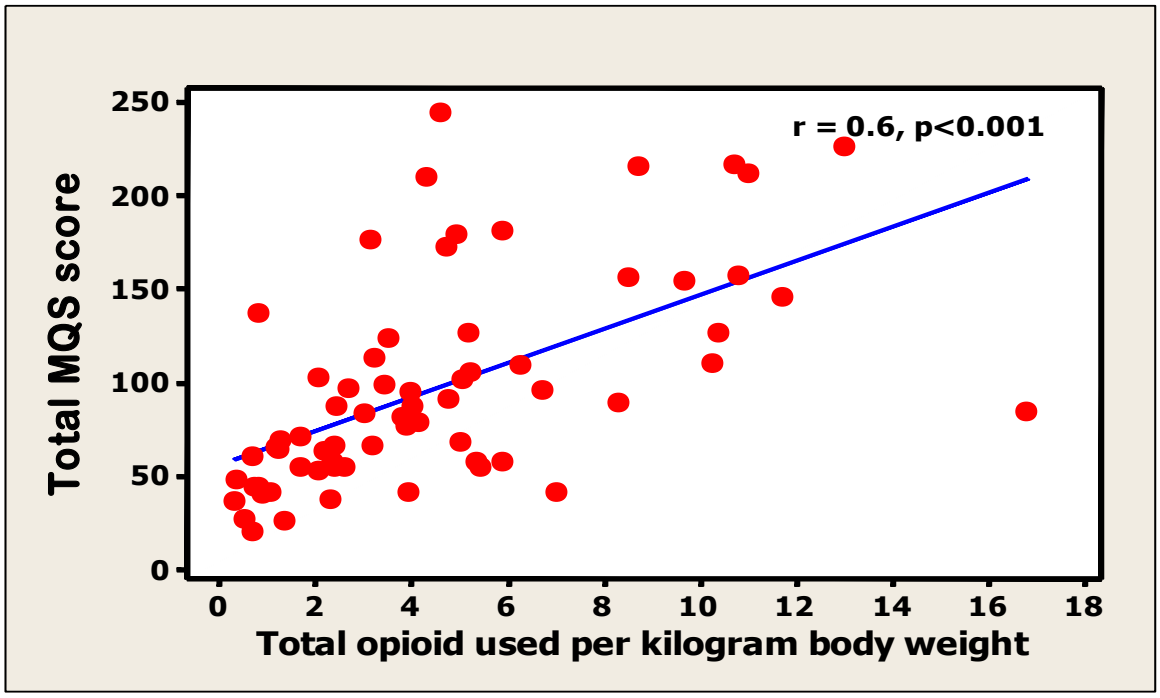


Figure 5.3: Correlation between total Analgesic Medication Quantification Scale score (MQS) and total opioid usage

Effect of $_{L}$-arginine supplementation on analgesic use during the course of hospital stay

Analgesic usage was similar in patients on arginine and placebo on day 1 with a mean analgesic medication quantification scale score (MQS) of 16.90 (95% CI, 14.40-19.40) and 17.77 (95% CI, 14.83-20.71), respectively, p = 0.647. From day 2 to 5, patients on arginine had lower MQS scores compared to their counterparts on placebo, and the difference became statistically significant on days 4 and 5, p = 0.001 and 0.011, respectively (Table 5.4).

Table 5.4: Daily Analgesic Medication Quantification Scale scores of patients on supplement

Day of assessment	Number of patients on arginine	Mean MQS (95% CI) for patients on arginine	Number of patients on placebo	Mean MQS (95% CI) for patients on placebo	p-value
Day 1	35	16.90 (14.40-19.40)	33	17.77 (14.83-20.71)	0.647
Day 2	33	18.70 (16.36-21.04)	32	21.31(18.39-24.23)	0.165
Day 3	28	17.17 (14.84-19.50)	30	20.46 (17.47-23.45)	0.084
Day 4	24	12.69 (10.54-14.84)	28	18.79 (15.82-21.76)	0.001
Day 5	16	10.72 (8.28-13.16)	25	17.48 (13.59-21.37)	0.011
Overall	35	73.35 (62.36-84.33)	33	120.00 (96.67-143.33)	0.001

Arginine usage was associated with a 38.9% reduction in the mean total dose of analgesics required during hospitalization of patients with severe VOC. The mean cumulative medication quantification score began to diverge after 24 hours of starting the treatment, with sustained separation of curves over the duration of the trial (Figure 5.3). The mean total MQS score for the arginine group was 73.35 (95% CI, 62.36-84.33) compared to 120.00 (95% CI, 96.67-143.33) recorded in the placebo group (p = 0.001) (Figure 5.4).

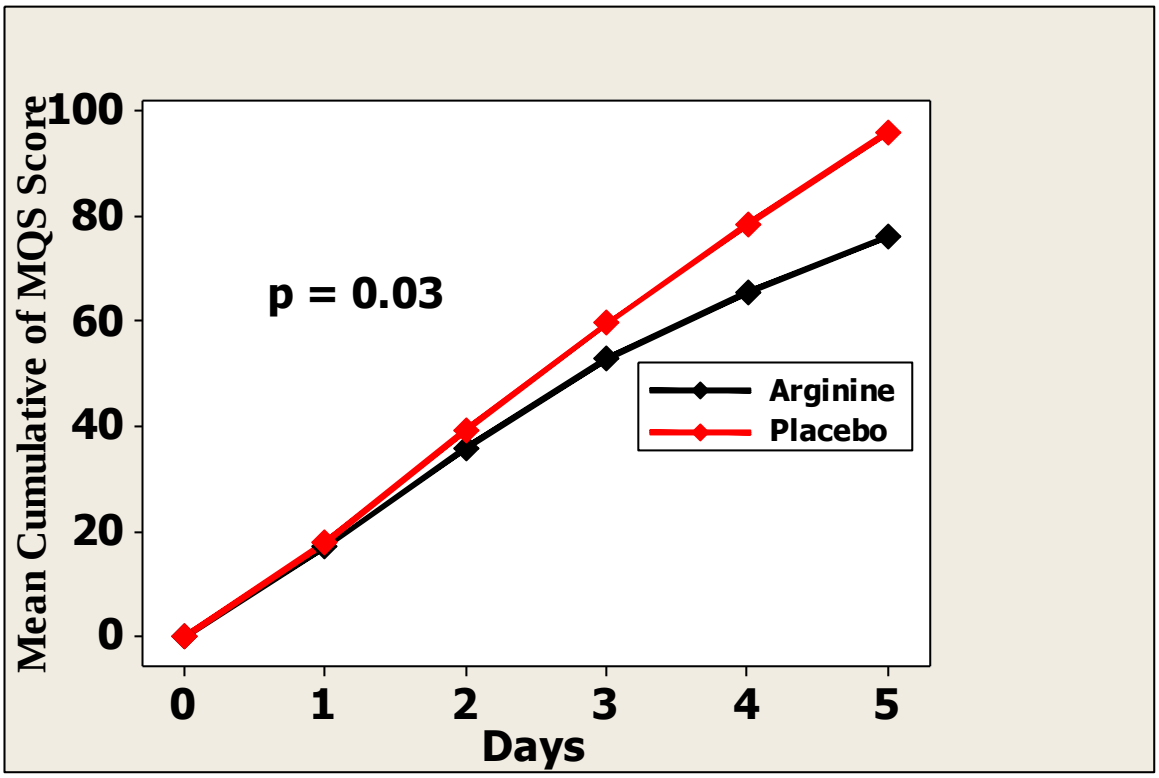


Figure 5.3: Line plot of cumulative medication quantification scale score (MQS) between the treatment arms showing a lower cumulative MQS in the arginine group, p = 0.03

With further categorisation of the analgesics into opioids and non-opioids, patients in the arginine arm had a 26.5% reduction in the total opioid requirement when compared to patients on placebo (Figure 5.5). The mean total dose of opioids used in the arginine group was 3.78 mg/kg (95% CI, 2.67-4.91) compared to 5.14 mg/kg (95% CI, 3.81-6.47) in the placebo group. The difference, though statistically insignificant (p = 0.115), is clinically relevant.

Analgesic requirement decreased by 39%, MQS score significantly lower in arginine (73) than placebo (120) arms, p = 0.001

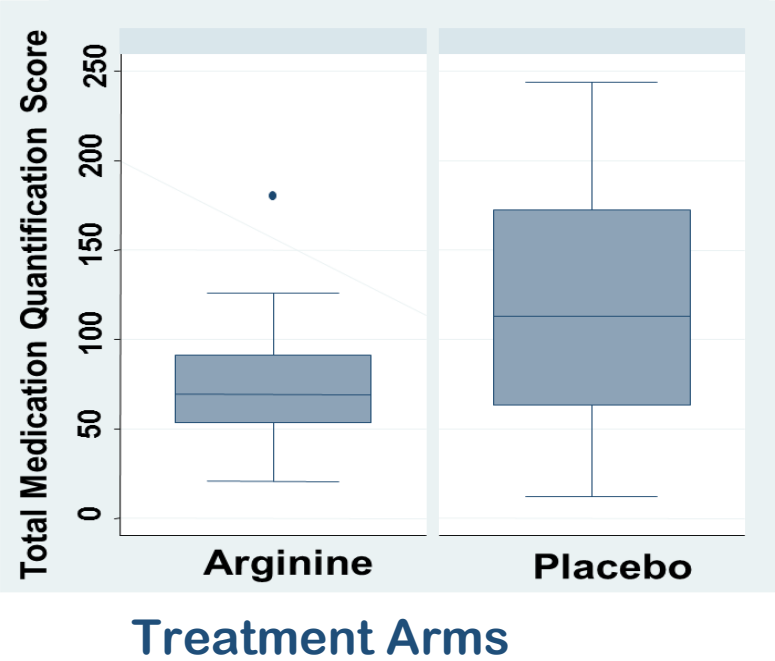


Figure 5.4: Box plot of the total medication quantification score showing the marked difference in the 25th, 50th and 75th percentile between the treatment arms

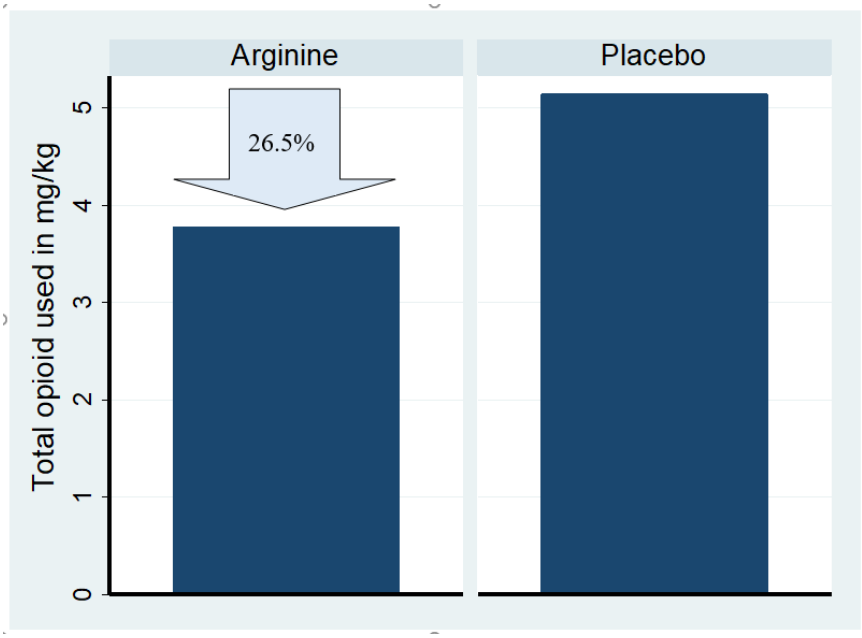


Figure 5.5: Box plot of total opioid usage (in mg/kg) between the treatment arms demonstrating a 26.5% reduction in the arginine group

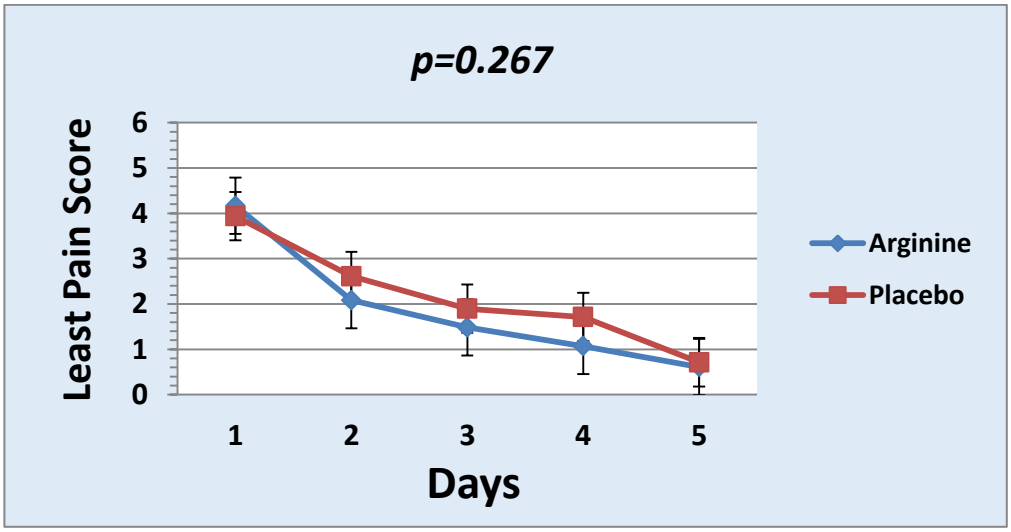
Secondary Outcomes

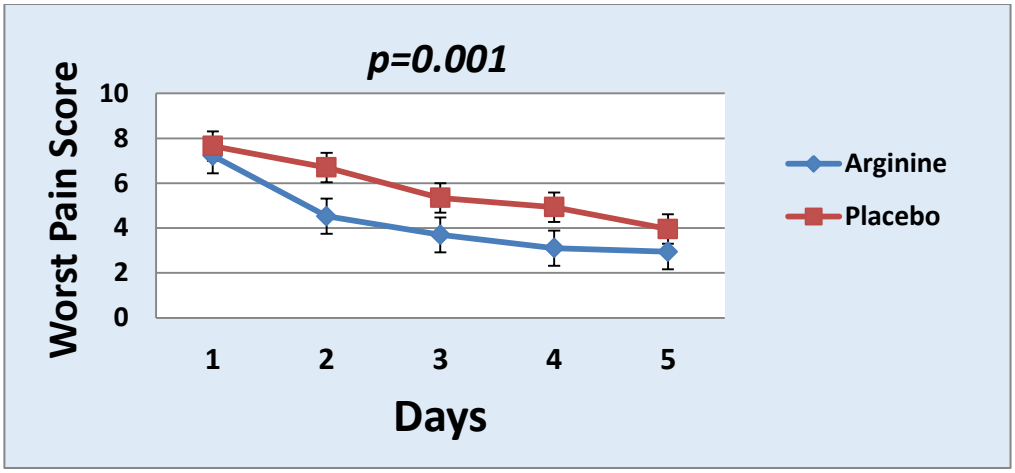
Impact of L-arginine supplementation on intensity of pains

The mean pain scores were similar at presentation: 8.72±1.13 (for arginine group) versus 8.42±1.26 (for placebo group) (p= 0.305) but became significantly lower for patients on arginine than for those on placebo, suggesting that arginine is associated with faster pain reduction than the placebo. The mean of the worst and the least pain scores reported by the patients are shown in Figure 5.6 which also shows the trend in the current pain. The decline in worst and least pain scores was more rapid in patients on the arginine arm compared to those in the placebo arm (Figure 5.6). The mean rate of decline in worst pain scores in the arginine arm was 1.50 /day (95% CI, 1.23-1.77) which is faster than the rate observed with placebo, 1.09 /day (95% CI, 0.94-1.24), p = 0.009.

Effect of L-arginine supplementation on the number of pain sites

The numbers of pain sites were similar on presentation. The most frequent number of pain sites was 2 in both supplement arms: 37.1% in the arginine arm versus 39.4% in the placebo arm (p = 0.599). By day 3, only 8.6% in the arginine arm as against 37.5% in the placebo arm complained of pains in two sites (p = 0.022). Of the 10 patients that complained of pains in multiple sites by day 5 of trial, only one was in the arginine arm compared to 9 in the placebo arm (p = 0.042).





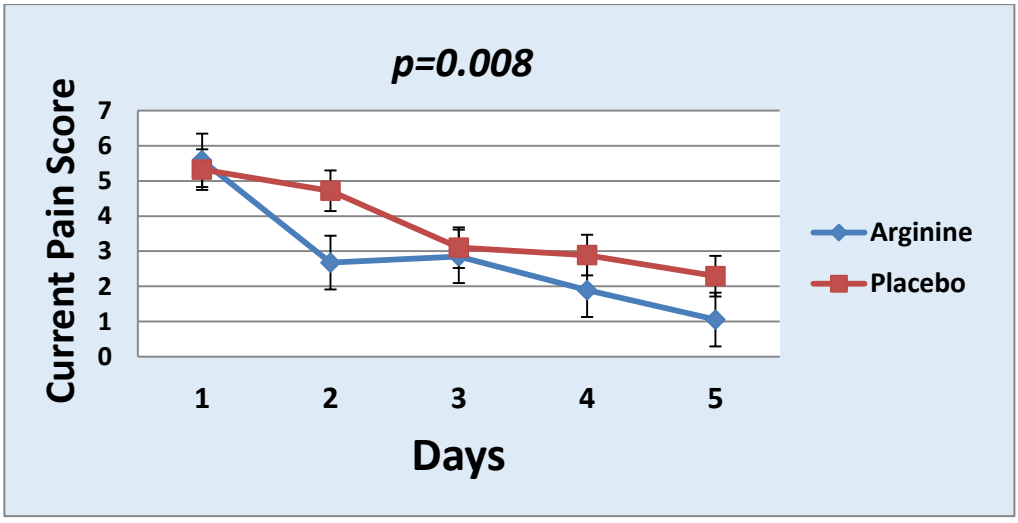


Figure 5.6: Trend in daily rate of decline in least, worst and current pain scores between the supplement arms showing mixed model p-value indicating difference in rate of decline

IMPACT OF ARGININE THERAPY ON THE ARGININE METABOLOME

Effects of oral arginine supplementation on plasma arginine and global arginine bioavailability

Supplementation with oral arginine was associated with a 124.6% (95% CI, 61.76-187.41) increase in plasma arginine levels compared to 29.3% (95% CI, 0.08-58.41) increase recorded in the placebo group, p = 0.007 (Table 5.7). Similarly, patients in the arginine arm had a higher arginine: ornithine ratio, (p = 0.034). The percentage increase in global arginine bioavailability was significantly higher in patients that received arginine: 59.0% (95% CI, 19.97-98.02) compared to -2.1% (95% CI, -26.50-22.33) in the placebo group (p = 0.011).

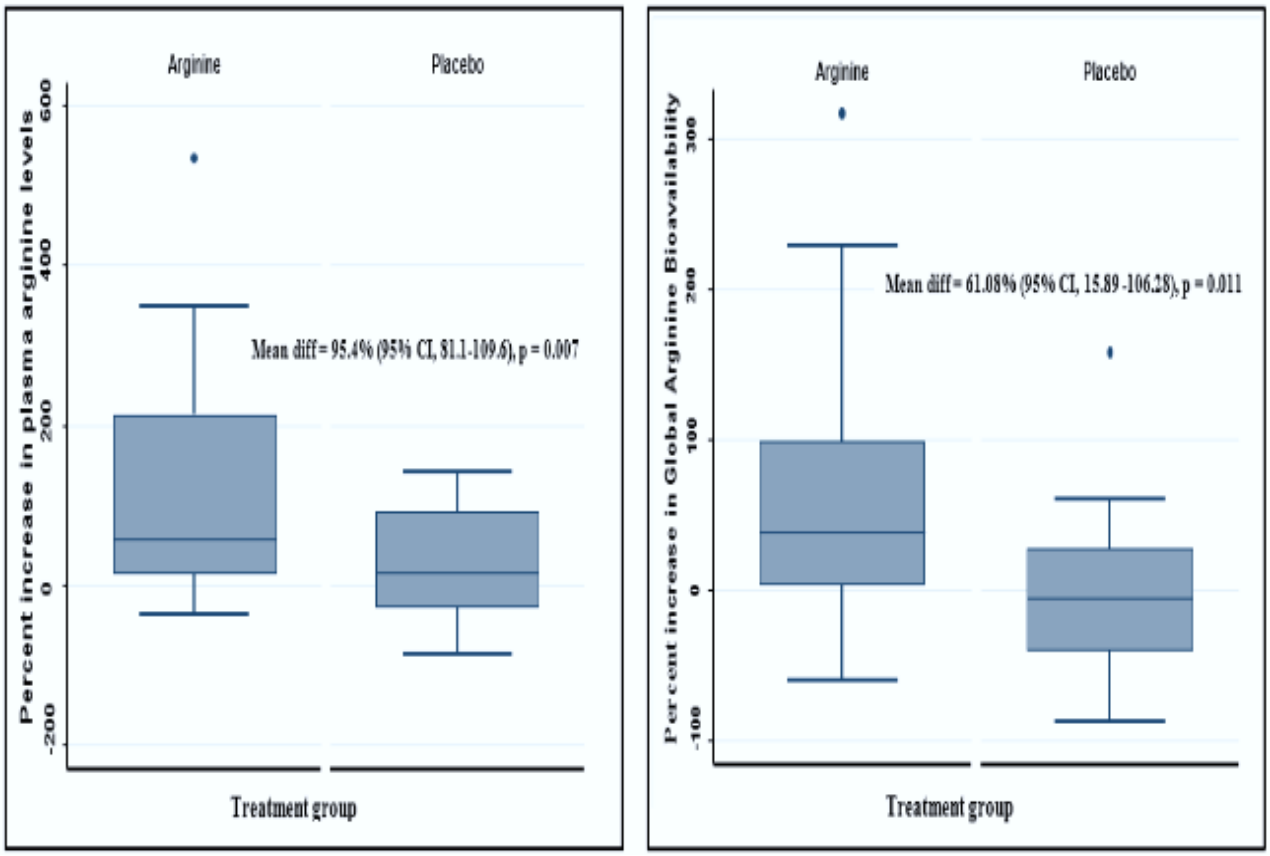


Figure 5.7: Oral arginine increases plasma arginine & global arginine bioavailability ratio (GABR).
The increase in plasma arginine levels was more profound in patients that received arginine. The difference in percentage increase in plasma arginine levels between the two treatment arms was 95.4%, p = 0.007. The graph on the left shows the percentage increase in the global arginine bioavailability ratio from baseline. Again, patients in the arginine treatment group had a more pronounced increase in GABR. A mean difference of 61.08% was recorded between the two treatment groups, p = 0.011.

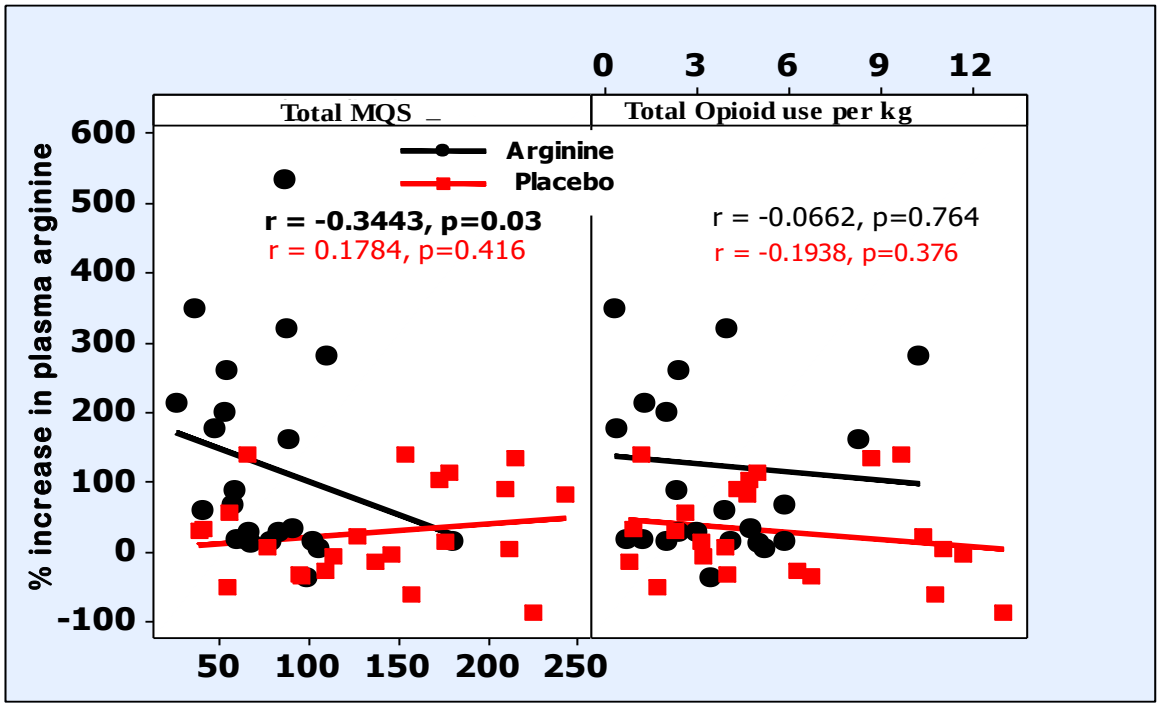
Table 5.5: Plasma arginine response to oral arginine therapy during vaso-occlusive crisis

Amino acid type	**Stage of study**	**Arginine group** **Mean±SE (95%CI)**	**Placebo group** **Mean±SE (95%CI)**	**p-value**
Arginine	Pre-supplement, µmol/L	88.49±8.18 (71.60, 105.34)	107.65±9.95 (87.02, 128.27)	
	Post-supplement, µmol/L	179.45±17.50 (143, 215.57)	126.07±13.75 (97.55, 154.58)	**0.007**
	Percentage increase, %	124.57±30.29 (61.76, 187.41)	29.25±14.06 (0.08, 58.41)	
Ornithine	Pre-supplement, µmol/L	256.36±40.98 (171.59, 341.13)	219.80±37.03 (142.78, 296.82)	
	Post-supplement, µmol/L	380.08±49.05 (278.63, 481.54)	267.19±39.01 (186.06, 348.33)	0.196
	Percentage increase, %	111.52±40.01 (28.54, 194.50)	69.16±30.11 (6.71, 131.61)	
Citrulline	Pre-supplement, µmol/L	13.95±6.71 (11.18, 16.72)	12.66±6.37 (10.21, 15.71)	
	Post-supplement, µmol/L	14.96±8.51 (11.45, 18.47)	14.09±6.23 (11.40, 16.79)	0.557
	Percentage increase, %	37.12±15.25 (5.49, 68.75)	24.98±15.24 (-6.62, 56.57)	
Arginine:Ornithine ratio	Pre-supplement	0.48±0.08 (0.33, 0.64)	0.56±0.07 (0.41, 0.707)	
	Post-supplement	0.55±0.06 (0.43, 0.68)	0.52±0.07 (0.38, 0.67)	**0.034**
	Percentage increase, %	45.73±15.06 (14.49, 76.96)	7.30±15.64 (-25.14, 39.74)	
Global Arginine Bioavailability Ratio	Pre-supplement	0.44±0.32 (0.31, 0.58)	0.52±0.29 (0.39, 0.64)	
	Post-supplement	0.52±0.27 (0.41, 0.64)	0.49±0.31 (0.36, 0.63)	**0.011**
	Percentage increase, %	59.0±18.82 (19.97, 98.02)	-2.08±11.74 (-26.50, 22.33)	

Correlation of increase in plasma arginine, global arginine bioavailability with analgesic usage during a painful crisis episode

As shown in Figure 5.8, percentage increase in plasma arginine level and global arginine bioavailability had a negative correlation with total medication quantification scale (MQS) score and opioid requirement for a vaso-occlusive episode. The correlation was more obvious among patients that received arginine and had significant increase in global arginine bioavailability. Total MQS score trended to consistently correlates (negative correlation) with percentage increase in plasma arginine and GABR, $r = -0.3354$, $p = 0.03$ and $r = -0.3225$, $p = 0.0847$, respectively, while total opioid requirement showed such relationship with percentage increase in GABR only, $r = 0.3445$, $p = 0.0205$.

A.



B.

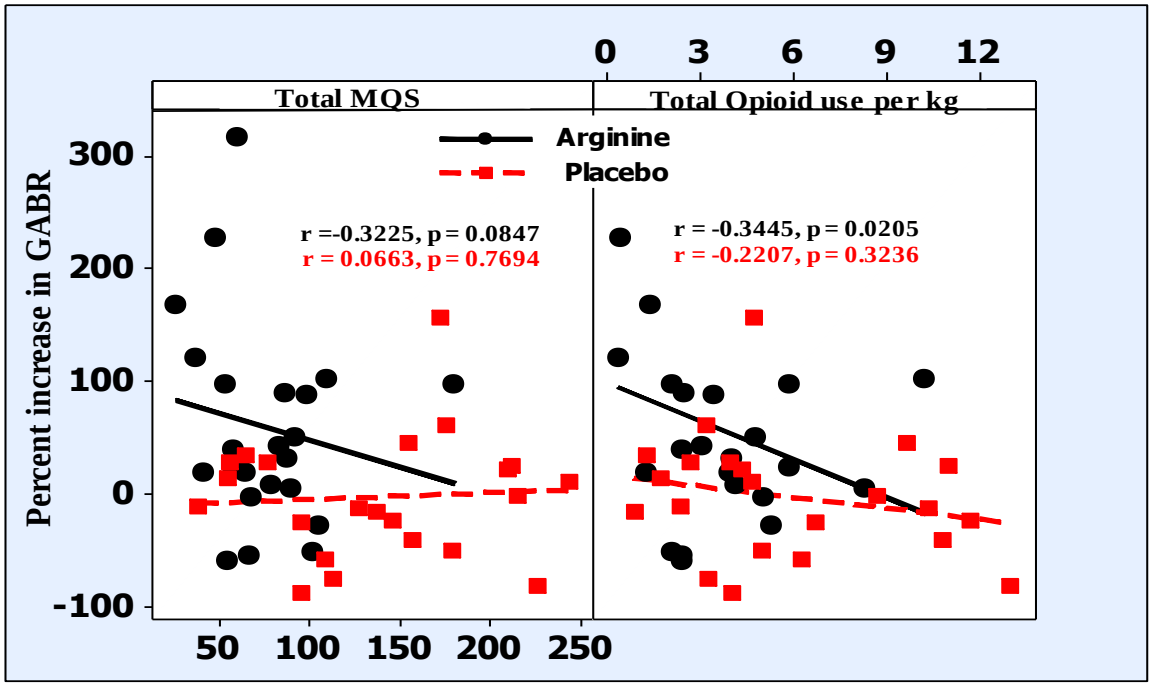


Figure 5.8: Correlation between percentage increase in plasma arginine (arg), global arginine bioavailability ratio (GABR), analgesic medication quantification scale (MQS) score and total opioid use in patients with vaso-occlusive crisis. Percentage increase in plasma arginine inversely correlated with total MQS in patients that received arginine, p = 0.03 (panel A). Total MQS tended to have negative correlation with percentage increase in GABR, p = 0.0847. The inverse relationship between percentage increase in GABR and total opioid requirement is also demonstrated in panel B which shows a significant correlation between them, p = 0.0205.

Time-to-resolution of vaso-occlusive pain episodes

The overall incidence of resolution of crisis over a 5-day period was 4.6 per 1000 child hours with a median time to resolution being 120 hours. The incidence of crisis resolution in patients on arginine was 6.4 per 1000 child hours compared to 3.1 per 1000 child hours in patients in the placebo group. A more rapid crisis resolution (resolution within 72 hours) was recorded in 25% of patients on arginine. On the contrary, it took 120 hours for 25% of those on placebo to achieve crisis resolution. A Log rank test of the difference in crisis resolution between the treatment arms proved to be statistically significant, Log rank Chi square = 5.27, p = 0.022 (Figure 5.9).

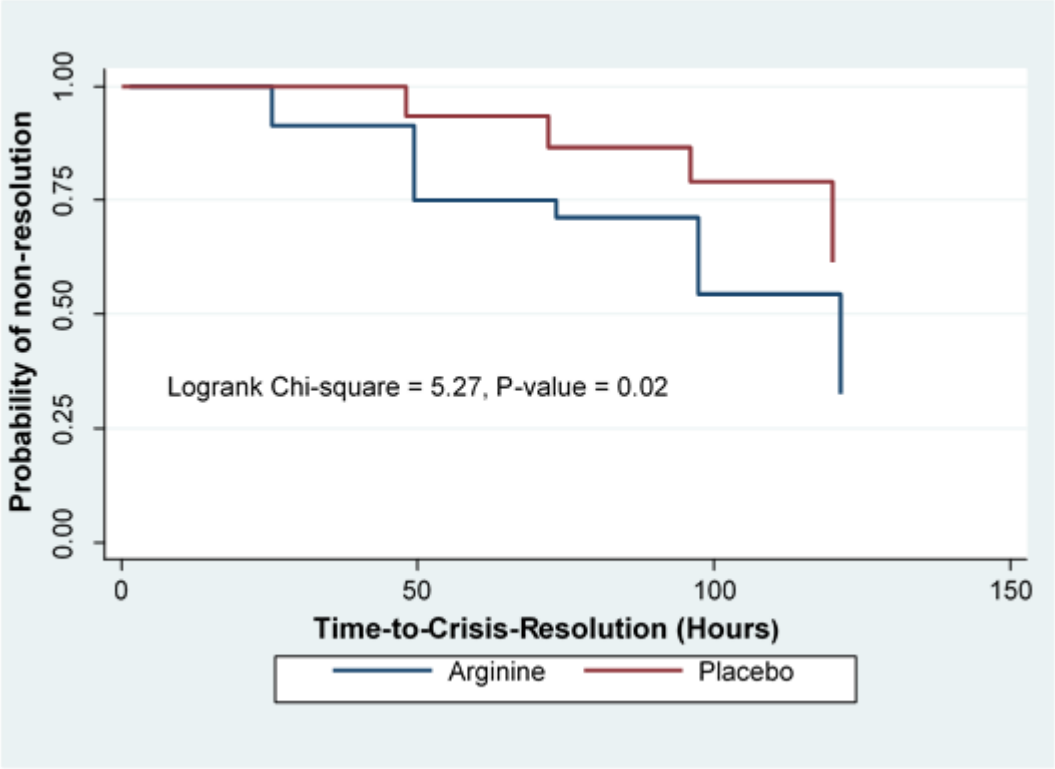


Figure 5.9: Kaplan-Meier plots showing time-to crisis resolution of shorter hours in the arginine group than in the placebo group

Length of hospital stay

The mean length of hospital stay was 109.91 (95% CI, 93.22-126.61) hours in the arginine group and 156.21 (130.34-182.08) hours in the placebo group (p = 0.003) with a mean

difference of 46.3 (95% CI, 16.44-76.16) hours. The median length of hospital stay was 105.00 hours (IQR, 72-144) for patients that received arginine and 141.00 hours (IQR, 117.00-205.00) for patients in the placebo group (Log rank test p = 0.002) (Figure 5.10).

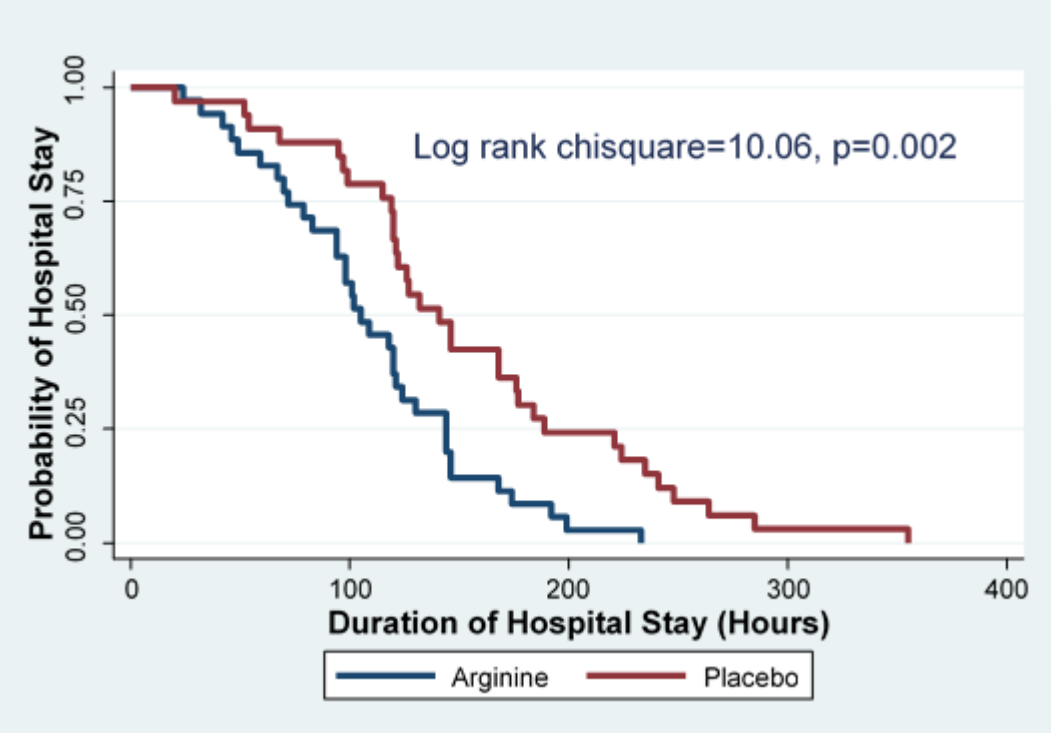


Figure 5.10: Kaplan-Meier plots of the duration of hospital stay by intervention group showing patients on arginine having shorter length of hospital with a median of 105 hours as against 141hours in the placebo group

Correlation between length of hospital stay and analgesic medication quantification score

There was a strong positive correlation between the length of hospital stay and analgesic medication usage: r = 0.77, p = 0.00 (Figure 5.11), signifying that patients' length of stay in the hospital was determined by the pain intensity.

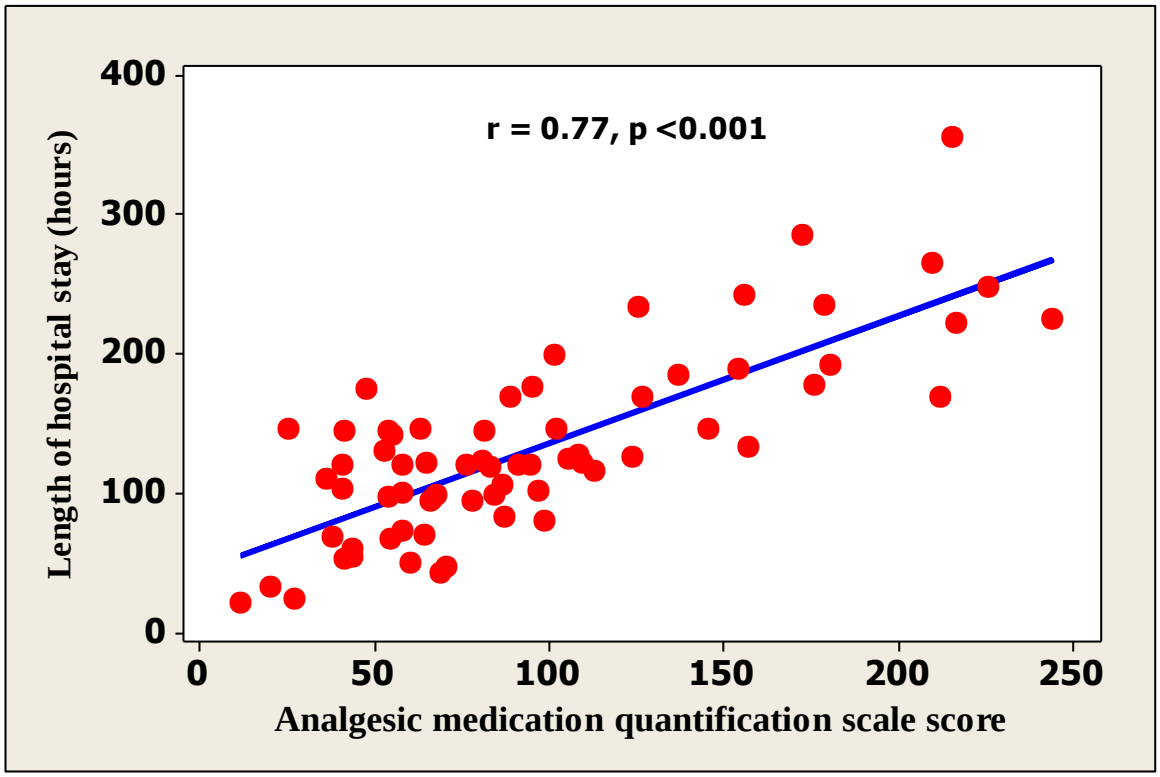


Figure 5.11: Scatter plot showing a strong positive correlation between length of hospital stay and analgesic medication quantification scale score, r = 0.77, p <0.001.

Readmission within one week of discharge

Readmission rate was slightly higher in the arginine group. Four (11.4%) of the patients that had arginine were readmitted within 7 days of hospital discharge as against three (9.1%) in the placebo group. The between-group difference was however small and insignificant, Fisher's p-value >0.99.

Concomitant medications

Various medications were used concomitantly with the research product. These were drugs routinely used in the management of patients with sickle cell crisis and included non-steroidal anti-inflammatory drugs, opioid analgesics, intravenous fluids, antimalarial drugs and antibiotics. The drugs used in the study patients and the frequencies of usage were comparable in the arginine and placebo arms.

Safety

The rate of adverse events was similar in both treatment groups: 71.4% in the arginine group versus 78.8% in the placebo group, p = 0.791. Four cases of serious adverse events were recorded in patients on placebo. Two were cases of empyema thoracis, one case of osteomyelitis and one death which occurred on the second day of admission. The mortality case occurred immediately after an intravenous (IV) bolus of diazepam, probably due to respiratory depression. The patient had severe acute chest syndrome, severe anaemia and was desaturating. The diazepam bolus was given to calm the patient by an intern who wanted to secure another intravenous route for blood transfusion but couldn't due to the patient's restlessness. There was however no serious adverse event in patients that received arginine (Table 5.6).

Adverse events with a higher incidence in the $_{L}$-arginine group than in the placebo group included vomiting (20.0% versus 3.0%, p = 0.068), restlessness and hiccups (2.9% versus 0%, p > 0.99). Other reported side effects occurred with higher incidence in the placebo group and included fever, abdominal pain and further deterioration in PCV warranting blood transfusion.

Table 5.**6**: Profile of adverse events reported during the supplementation period

Adverse Events	**Arginine group (n=35)** *No. of patients (%)*	**Placebo group (n=33)** *No. of patients (%)*	**Test statistics**	**p-value**
Gastrointestinal disorders				
Vomiting	7 (20.0%)	1 (3.0%)	Fisher's Exact	0.068
Nausea	1 (2.9%)	0	Fisher's Exact	>0.999
Diarrhoea	1 (2.9%)	0	Fisher's Exact	>0.999
Abdominal pain	2 (5.7%)	4 (12.1%)	Fisher's Exact	0.410
Nervous system disorders				
Headache	1 (2.9%)	2 (6.1%)	Fisher's Exact	0.598
Restlessness	1 (2.9%)	0	Fisher's Exact	>0.999
Respiratory disorders				
Hiccups	1 (2.9%)	0	Fisher's Exact	>0.999
Cough	0	1 (3.0%)	Fisher's Exact	0.471
Empyema thoracis	0	2 (6.1%)	Fisher's Exact	0.218
Musculoskeletal disorders				
Osteomyelitis	0	1 (3.0%)	Fisher's Exact	>0.999
Haematological disorders				
Significant fall in PCV	5 (14.3%)	5 (15.2%)	Fisher's Exact	0.840
General disorder				
Fever	5(14.3%)	6(18.2%)	Fisher's Exact	0.587
Rashes(+bullous eruption)	1(2.9%)	3(9.1%)	Fisher's Exact	0.336
Death	0	1 (3.0%)	Fisher's Exact	0.471

Adverse events are categorized by system organ class and preferred terms in line with the Medical Dictionary for Regulatory Activities, version 12.0. PCV = packed cell volume

5.2 DISCUSSION

The success of pain management during sickle cell crisis depends on prompt and adequate assessment of pain intensity, number of pain sites and judicious analgesia. In the present study, the intensity of pain and multiplicity of pain sites were similar across the various age groups in children, a finding that is congruent with the report of Jacob *et al*[162] among children with sickle cell disease. The intensity of pain strongly correlated with the number of pain sites similar to the report of McClish *et al*[163] in adults with sickle cell disease.

Although clinical trials in humans with painful conditions have yielded contradictory results with arginine supplementation, more studies have affirmed that arginine administration is associated with a reduction in pain.[27,164-167] NO-related vasodilation from arginine supplementation may contribute to pain relief. In addition, arginine is also a precursor of kyotorphin (L-tyrosyl-L-arginine), which is an analgesic dipeptide.[166] Marked analgesia was reported in non-SCD patients with various forms of pain after 30-40 minutes of arginine supplementation compared to placebo, with an effect that lasted 6-24 hours. Interestingly, the L-arginine-induced pain relief was dose-dependent and blocked by intravenous naloxone. A dose-dependent impact on NO metabolite production in children with VOC has been reported with oral arginine,[35,90] and more recently, with intravenous arginine with respect to mitochondrial function and oxidative stress.[161] In the present study, arginine supplementation increased the rate of pain intensity reduction and enhanced early achievement of analgesia with conventional analgesics compared to the placebo. The rate of pain reduction barely exceeded 1 point per day on the pain score when using only conventional analgesics compared to the 1.5 point per day reduction recorded with addition of arginine to the treatment protocol, thus implying an additional 37% increase in analgesia. A decrease in the frequency and intensity of chest pain has been reported by Bortolotti *et al*[164] in adult patients with eosophageal motor disorders following oral administration of arginine, thus further corroborating the effects of arginine in management of pain. Similarly, other authors have observed improved analgesia when arginine was co-administered with conventional analgesics in postoperative dental pain relieve[167] and in sickle cell patients with vaso-occlusive pain episodes.[27]

The total analgesic medication quantification scale score, an index that captures all the analgesic drugs (opioids and non-opioids) used in the treatment of a painful episode was significantly lower in the arginine group than in the placebo group, implying analgesic sparing

effect of arginine in pain management. This effect of arginine has previously been documented by Morris *et al*[27] in the setting of sickle cell disease children with painful crises, where a 54% reduction in opioid use was observed. Although, a 54% reduction in opioid use was not achieved in the current study, the reduction in opioid requirement by 26.5% still corroborates the position of Morris *et al*. The Morris *et al*[27] study utilised parenteral arginine, whereas oral arginine was used in the current study which may account for the difference in narcotic requirement between these two studies.

The relevance of the analgesic sparing property of arginine was further demonstrated by the faster crisis resolution and by extension, the shorter hospital stays of patients who had arginine compared to placebo in the present study. A mean difference of 46.3 hours in length of hospital stays recorded between the arginine group and the placebo group has both clinical and economic relevance in contemporary patient care. This finding was corroborated by the report of Morris *et al*[27] who described a 17-hour reduction in length of stay for those that received arginine along with conventional analgesic medications. It is however not certain why the difference in length of hospital stays in our study almost tripled that of Morris *et al*. Differences in hospitalisation protocols and patient discharge policies may have played at least a minimal role. A large-scale multicentre trial may further clarify the role of arginine in pain management and in the reduction in length of hospital stay in patients with sickle cell painful crisis and further provides the basis for advocating for the incorporation of $_{L}$-arginine into the standard protocol for the management of vaso-occlusive pain episodes in sickle cell patients.

The safety profile of this study is comparable to those of Morris *et al*[92] and other authors[168,169] who also documented mainly minor side effects following administration of arginine. Vomiting was a prominent side effect in the arginine group in the current study but this did not reach statistical significance. There was no case of serious adverse event in the arginine arm. All the four cases of serious adverse events recorded were in the placebo group.

Finally, the specific mechanism(s)-of-action for arginine therapy remains to be determined, although several feasible mechanisms that include NO generation, improvement in mitochondrial function/bioenergetics[164] and decreased oxidative stress have been described, in addition to NO-independent mechanisms specifically targeting pain pathways that impact kyotorphin production.[162] It is interesting to note that the recently FDA-approved amino acid $_{L}$-

glutamine is an arginine precursor whose mechanism remains unclear, but likely shares some common pathways, particularly the antioxidant effect. $_{L}$-Glutamine increases nicotinamide adenine dinucleotides redox potential in sickle red blood cells and reduces oxidative damage by increasing the availability of reduced glutathione.[170] Clinically, these biochemical effects manifested as a lower incidence of vaso-occlusive crises, a lower rate of hospitalizations and shorter hospital stays for patients eventually hospitalised for VOC in a pivotal phase 3 clinical trial[170] conducted by Emmaus Medical, Inc. Unlike arginine, $_{L}$-glutamine is being advocated mainly for VOC prophylaxis. Hydroxyurea, the first FDA-approved drug for treatment of sickle cell disease also reduces the frequency of crises and the need for blood transfusions. However, like $_{L}$-glutamine, hydroxyurea is mainly for crises prevention through its ability to increase the production of foetal haemoglobin and reduction of red blood cell adhesion receptor expression.[171] Even then, some patients do not respond to hydroxyurea, and there are serious safety concerns as well, so there is still a huge unmet need in this disease for new therapies, beyond the conventional analgesics, targeting symptoms' control in patients hospitalised for acute pains due to severe VOC.

5.3 SUMMARY OF MAJOR FINDINGS

This study has shown that arginine given orally impacts positively on the control of pain in children with painful sickle cell crisis. The pain intensity reduces faster while the analgesic requirement is lesser in the arginine arm. Oral arginine is safe, well tolerated by patients with sickle cell crisis and contributes to a shorter hospital stay.

CHAPTER 6:

SUMMARY AND CONCLUSIONS

This concluding chapter of this thesis highlights the overall aim of the thesis, summarizes key objectives, hypothesis, results, strength and limitation of the thesis.

6.1 Summary of the aim of thesis

The main aim was to determine the effect of sickle cell crisis on the myocardium already affected by chronic sickle cell anaemia and explore the possible impact of arginine supplementation on the cardiovascular complications of sickle cell crisis. In addition, the demonstration of the effect of arginine on pain management and duration was intended.

6.2 Summary of the objectives, hypotheses and important findings

Objectives	Hypotheses	Key findings
To determine the effect of sickle cell crisis on cardiovascular parameters of SCA children.	Sickle cell crisis negatively influences cardiovascular parameters of children with sickle cell anaemia.	Sickle cell crisis increases the heart rate and blood pressure. ECG evidence of myocardial ischaemia was more prevalent in patients in crisis. The prevalence of LV and RV dysfunctions is tripled in sickle cell crisis. The prevalence of pulmonary hypertension is also tripled among those with sickle cell crisis. Cardiac biomarker levels are elevated in sickle cell crisis, and the elevation was more pronounced in

		children with acute chest syndrome.
To evaluate the impact of arginine supplementation on cardiac changes in VOC and ACS.	Arginine supplementation has significant impact on cardiac changes in vaso-occlusive crisis and acute chest syndrome.	Arginine therapy was associated with a 21.5% reduction in mean pulmonary artery pressure. The myocardial ischaemia score was much lower with arginine supplementation while the placebo made no changes to the ischaemia score. Arginine usage reduces the prevalence of ventricular dysfunction and cardiac biomarker levels.
To determine the role of arginine as adjuvant therapy in the management of pain in sickle cell vaso-occlusive crisis.	Arginine as adjuvant therapy has a significant role in management of pain in sickle cell vaso-occlusive crisis.	The pain intensity reduces faster while the analgesic requirement is lesser when arginine is used with conventional analgesics. Oral arginine is safe, well tolerated by patients with sickle cell crisis and contributes to a faster crisis resolution and shorter hospital stay.

6.3 Strengths of the thesis

The study utilised comparative case-control design to establish that sickle cell crisis is a stressor to the heart that is already working at a maximally compensated state using a myocardial ischaemic score developed in Nigeria along with cardiac biomarkers, thus demonstrating more convincingly that ischaemic changes are common during episodes of painful crisis.

Although it is known that pulmonary hypertension can complicate sickle cell anaemia, this study demonstrated that sickle cell crisis increases the prevalence and severity of pulmonary hypertension.

Many authors have reported conflicting data on the prevalence of ventricular dysfunction in sickle cell disease. This study, utilising both load-dependent and load-independent echocardiographic parameters along with biochemical markers further strengthened the opinion that cardiac dysfunction may complicate sickle cell disease, at least, at sub-clinical level.

This is the first study employing detailed echocardiographic and electrocardiographic evaluations to determine the effect of sickle cell crisis in children with sickle cell anaemia in Nigeria.

Furthermore, the study provides insight into the potential usefulness of arginine in the treatment of the multi-systemic manifestations of sickle cell crisis in children in Nigeria. This, to the best of author's knowledge, is the first study evaluating the effects of arginine in sickle cell crisis in sub-Saharan Africa.

The study, demonstrated a more rapid resolution of crisis-related cardiac abnormalities, bone pain and shorter hospitalisation time associated with administration of oral arginine. It is also the first study to assess the effects of arginine on the cardiovascular and musculoskeletal systems concurrently. An additional first is the successful utilisation of oral arginine in children with vaso-occlusive painful crisis in Africa and demonstrated a significant increase in plasma arginine and global arginine bioavailability despite a widely acclaimed high hepatic first pass effect.

6.4 Limitations of the thesis

- No long-term follow up of patients following crises to assess echocardiographic changes in the steady state following crisis.
- No facility to undertake direct intracardiac catheter measurements such as pulmonary artery pressure.
- Indirect assessment of pulmonary artery pressures and calculation of pulmonary vascular resistance and other cardiac indices using echocardiographic-acquired parameters which may have many shortcomings.
- Some patients have poor image quality which could not be improved upon despite application of standard echocardiographic protocols.
- Although, PVR determined by echocardiography correlates well with values obtained from cardiac catheterisation, anatomic modification of right heart structures may make accurate measurements of the RVOT VTI difficult. In patients with severe PVR, this method may also be inaccurate. Therefore, cardiac catheterization remains the gold standard in determining PVR.
- The effect of respiration on cardiac parameters' measurements could not be adequately controlled as some of the younger patients will not consistently obey the command to hold their breath at end-expiration.
- The study could not differentiate if the cardiac changes were of recent onset or a cumulative effect of previous ischaemic injuries.
- The study was conducted at only two sites within Nigeria and involved few patients, which may limit the capacity to generalise the findings.
- The pain scale used is subjective, though validated, and patients' ability to give accurate assessment of pain severity may be skewed by various cultural and psychosocial factors.
- This study did not include confounding variables such as variation in the level of arginine in patient's diet and differences in endogenous arginine production, which can affect the plasma arginine concentration.
- Changes in pain levels are assumed to be cofounded by inherent patient's pain threshold which was not measured.
- The proportion of children that were on hydroxyurea was too small to allow for meaningful statistical analysis.

- Length of hospital stay is likely to be confounded by other comorbid conditions such as their nutritional status or the presence of infectious diseases like malaria or HIV, all conditions that may impact global arginine bioavailability, and potential response to arginine therapy.
- Finally, the study did not explore the specific mechanism(s)-of-action for arginine therapy, a much needed scientific knowledge in contemporary therapeutics.

6.5 Further research

There are still many unanswered questions regarding the exact pathogenesis of cardiovascular changes in sickle cell crisis and the clinical relevance of these changes. Future research should be focused at unveiling these inadequately explored areas.

It is uncertain if the potential arginine effects demonstrated in patients with sickle cell vaso-occlusive crisis could translate into improved clinical care of patients with crisis in the near future and as such urgent need for a phase 3 multicentre trial on the cardiovascular benefits of arginine therapy in children with vaso-occlusive events as well as the relevance of arginine in pain management is advocated.

Serial studies using more advanced technologies like Magnetic Resonance Imaging to assess myocardial fibrosis as evidence of cumulative cardiac injuries with repeated sickle cell crises may be needed to fully characterise the effects of sickle cell crisis on cardiac parameters.

The mechanism(s) of arginine action remains unclear and further investigation in this regard is warranted.

Finally, it will be necessary to establish the effect of early administration of oral arginine, at the first sign of crisis, on the emergency unit visits and hospitalisation of children with sickle cell disease.

6.6 CONCLUSIONS

This thesis shows that sickle cell crisis is a stressor to the cardiovascular system. The systemic blood pressure and the pulmonary artery pressures are increased by crisis, particularly the vaso-occlusive painful episode and the acute chest syndrome. Besides the blood pressure changes, sickle cell crisis increases the prevalence of conduction abnormalities, ST-segment elevation and myocardial ischaemia. Furthermore, children with sickle cell crisis have higher incidence of right and left ventricular dysfunctions as well as elevation of plasma NT-proBNP and troponin I levels.

Administration of oral arginine to patients having vaso-occlusive pain episode and acute chest syndrome raises the plasma arginine and hence the global arginine bioavailability to levels sufficiently high to produce significant effects in the cardiovascular system.

Children with high tricuspid regurgitation velocities have lower plasma arginine levels and lower global arginine bioavailability ratio. Similarly, high mean pulmonary artery pressure was associated with a lower plasma arginine level and a lower global arginine bioavailability ratio.

Arginine therapy reduces the systolic and diastolic blood pressures by 5.29 mmHg and 4.54 mmHg, respectively, while the mean pulmonary artery pressure is reduced by 21.5%.

The prevalence of first degree heart block (prolonged PR intervals), interventricular conduction delay and myocardial ischaemia is reduced following arginine therapy. Right and left ventricular dysfunctions subsided with arginine supplementation and the plasma NT-proBNP and troponin I levels decreased with arginine administration. Therefore, arginine supplementation holds a promising benefit that will warrant further large scale phase 3 clinical trial specifically targeted at establishing the beneficial effects of arginine on the cardiovascular changes in children with sickle cell crisis.

Interestingly, the study shows that the effect of arginine goes beyond the cardiovascular changes in sickle cell crisis. Pain, being the main symptom in vaso-occlusive pain crisis, is relieved faster when conventional analgesics are used along with arginine. Overall, arginine reduces the analgesic requirements per pain episode by 39%. The analgesic effect of arginine translates into a shorter time-to-crisis resolution and a shorter duration of hospital stay.

Arginine, a nutritional supplement, is safe and well tolerated by children with sickle cell vaso-occlusive crisis and acute chest syndrome. When used at appropriate doses, the nutrient has the potential of revolutionising the care of sickle cell patients admitted with severe vaso-occlusive crisis in Nigeria. Therefore, a large scale multicentre study of arginine in a subset of sickle cell disease patients with vaso-occlusive pain events and acute chest syndrome is strongly advocated.

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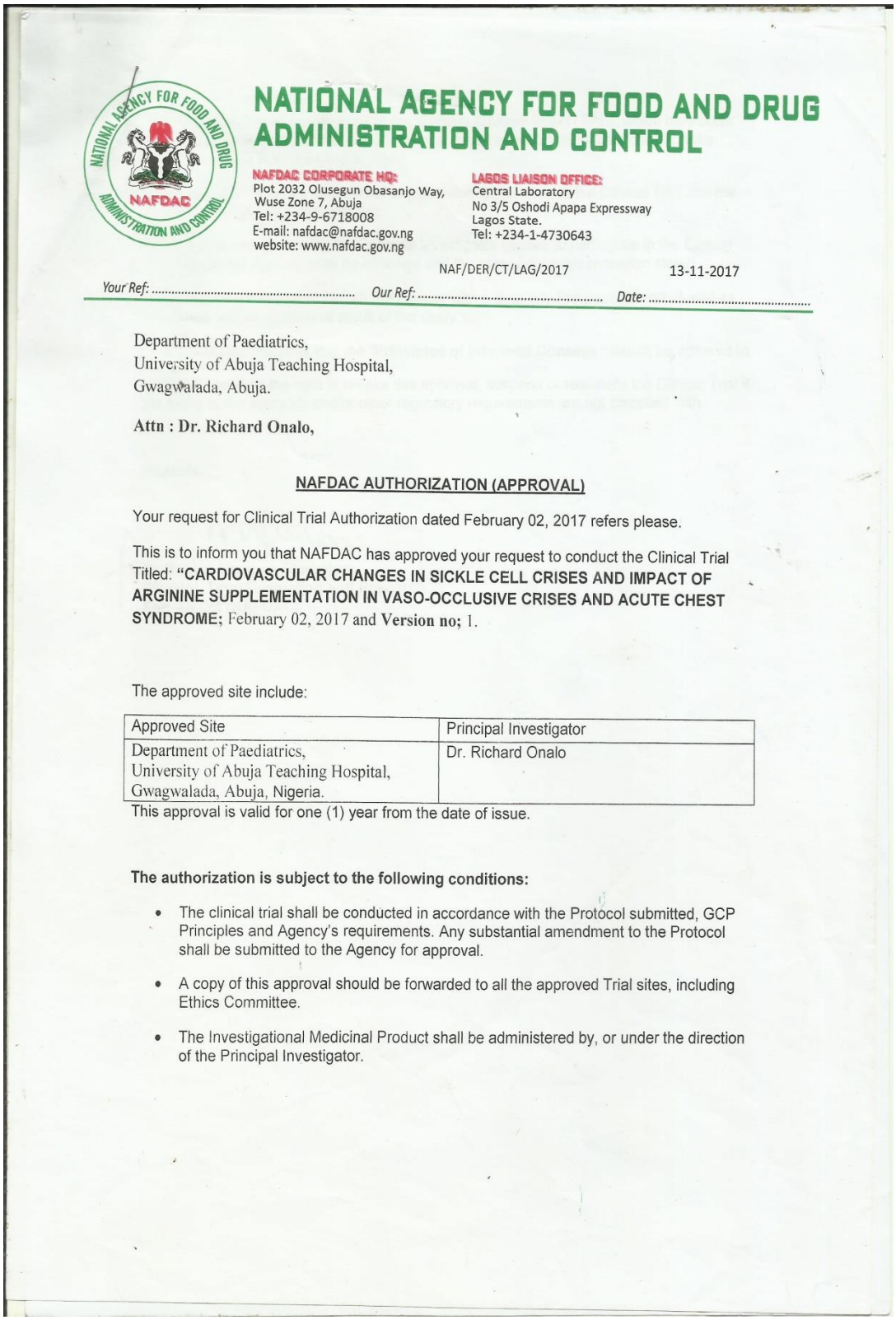
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APPENDIX 1: Ethical Clearance Certificates: NAFDAC, HREC-(WITS, UATH, NHA),AIICO



NATIONAL AGENCY FOR FOOD AND DRUG ADMINISTRATION AND CONTROL

NAFDAC CORPORATE HQ:
Plot 2032 Olusegun Obasanjo Way,
Wuse Zone 7, Abuja
Tel: +234-9-6718008
E-mail: nafdac@nafdac.gov.ng
website: www.nafdac.gov.ng

LAGOS LIAISON OFFICE:
Central Laboratory
No 3/5 Oshodi Apapa Expressway
Lagos State.
Tel: +234-1-4730643

Your Ref: ..... Our Ref: NAF/DER/CT/LAG/2017 Date: 13-11-2017

Department of Paediatrics,
University of Abuja Teaching Hospital,
Gwagwalada, Abuja.

Attn : Dr. Richard Onalo,

NAFDAC AUTHORIZATION (APPROVAL)

Your request for Clinical Trial Authorization dated February 02, 2017 refers please.

This is to inform you that NAFDAC has approved your request to conduct the Clinical Trial Titled: **"CARDIOVASCULAR CHANGES IN SICKLE CELL CRISES AND IMPACT OF ARGININE SUPPLEMENTATION IN VASO-OCCLUSIVE CRISES AND ACUTE CHEST SYNDROME;** February 02, 2017 and **Version no;** 1.

The approved site include:

Approved Site	Principal Investigator
Department of Paediatrics, University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria.	Dr. Richard Onalo

This approval is valid for one (1) year from the date of issue.

The authorization is subject to the following conditions:

- The clinical trial shall be conducted in accordance with the Protocol submitted, GCP Principles and Agency's requirements. Any substantial amendment to the Protocol shall be submitted to the Agency for approval.
- A copy of this approval should be forwarded to all the approved Trial sites, including Ethics Committee.
- The Investigational Medicinal Product shall be administered by, or under the direction of the Principal Investigator.

- NAFDAC shall be informed of any Serious Adverse Event (SAE), which may occur during the Clinical Trial and of any data received which might cast doubt on the continuation of the Clinical Trial.
- NAFDAC shall be informed of any decision to discontinue the Clinical Trial and the reason for such cancellation.
- In the event whereby the Principal Investigator ceases to participate in the Clinical Trial, the Agency shall be informed and the reason for such cessation stated.
- You are required to furnish the Agency with a quarterly Progress Report as well as the Report of the final result of the study.
- NAFDAC requires that the "**Principles of Informed Consent** "should be adhered to.

NAFDAC reserves the right to revoke this approval, suspend or terminate the Clinical Trial if the terms of the approval and/or other regulatory requirements are not complied with.

Regards.

Dr Monica Hemben Eimunjeze
Director, Drug Evaluation and Research
For: Ag.DG (NAFDAC)

R14/49 Dr Richard Onalo

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160326

NAME: (Principal Investigator)	Dr Richard Onalo
DEPARTMENT:	Paediatrics University of Abuja Teaching Hospital, Abuja
PROJECT TITLE:	Arginine Supplementation in Children with Sickle Cell Anaemia Presenting with Severe Vaso-Occlusive Crisis and Acute Chest Syndrome
DATE CONSIDERED:	01/04/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof P. Cooper and Prof A. Cilliers
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/06/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated,from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review in March and will therefore be due in the month of March each year.

Principal Investigator Signature

Date 21/06/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF ABUJA TEACHING HOSPITAL

P.M.B. 228, ABUJA - F.C.T. NIGERIA
07040045614, 09-2905535, 09-2904040
www. uath. ng.org.

Chief Medical Director
Dr. Peter Alabi
BM. BCH, FMCP

Chairman Medical Advisory Committee
Dr. A.S. Haruna
MBBS, FWACP

Chairman, Board of Management
Valentine I. Attah Ph D

Director of Administration
Musa Abdullahi
MPA, AHAN

Our Ref: FCT/UATH/HREC/PR/517

Your Ref: _____

Date: 28/4/16

Dr Richard Onalo
Dept. of Paediatrics
UATH
F C T - Abuja

Letter of Approval

Proposed Title: Arginine supplementation in children with sickle-cell anaemia presenting with severe vaso-occlusive crisis and acute chest syndrome.

Proposed Site: UATH

Sponsor: Principal Investigator

Your submission to the committee on UATH Health Research Ethics Committee on the above named protocol refers.

The Committee reviewed the following documents:

- A completed UATH HREC Application form
- Informed Consent Form
- Research Proposal
- Questionnaire/Proforma

The committee has considered the ethical merit of your submission and approved the protocol. The approval is for one year and will lapse on 27/4/17. It can be renewed on request. Modification of any part of the research methodology will require an approval.

Accept assurances of our highest regards.

Prof. B Ekele
Chairman UATH HREC

NATIONAL HOSPITAL

(Established by Act No 36 of 1999).

Board Chairman

Chief Medical Director/CEO
Dr. J. A. F. Momoh, MBBC, MSC, FWACP(LM)

Director of Administration
Dr. Peter O. Egwakhide
P.hd, FCIA, FIPMA, FIHE, ANIM, ACIPM, AIHSAN, CIPS

Director of Clinical Services/CMAC
Dr. Oluseyi Oniyangi, MBBS, FWWACP (Paed), FIPNA

NHA/ADMIN/236/V.VII

17th February, 2017

RE: CARDOIVASCULAR CHANGES IN SICKLE CELL CRISIS AND IMPACT OF ARGININE SUPPLEMENTATION IN VASO-OCCLUSIVE CRISIS AND ACUTE CHEST SYNDROME NHA/EC/090/2016

Health Research Ethics Committee (HREC) Assigned number:	NHA/EC/090-/2016
Name of Principal Investigator:	Dr. Richard Onalo
Address of Principal Investigator	Department of Paediatrics University of Abuja Teaching Hospital Gwagwalada Abuja.
Date of Receipt of Valid Application:	28th, November 2016

Notice of Approval

This is to inform you that the research described in the submitted protocol, the consent forms, and other changes stated in the submitted research protocol addendum have been reviewed and *given full approval by the Institutional Review Board (IRB) Committee, National Hospital Abuja.*

This approval dates from **17th February, 2017** to **16th February, 2019** If there is delay in starting the research, please inform the HREC National Hospital Abuja so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study.*

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit to your research site without previous notification.

Dr. Oluseyi Oniyangi
(DCS/CMAC)
For: Chairman, HREC, National Hospital

Plot 132 Central District (Phase II) P.M.B. 425, Garki - Abuja Nigeria
Telephone: 0803-787-9543, 0809-751-9764, 0809-752-0012
E-mail: info@nationalhospitalabuja.net www.nationalhospitalabuja.net

23 November 2016

To Whom it May Concern:

RE: Cardiovascular changes in sickle cell crisis and impact of arginine supplementation in vaso-occlusive crisis and acute chest syndrome.

As project manager for the Pan African Clinical Trial Registry (www.pactr.org) database, it is my pleasure to inform you that your application to our registry has been accepted. Your unique identification number for the registry is **PACTR201611001864290**

Please be advised that you are responsible for updating your trial, or for informing us of changes to your trial.

Additionally, please provide us with copies of your ethical clearance letters as we must have these on file (via email, post or fax) at your earliest convenience if you have not already done so.

Please do not hesitate to contact us at +27 21 938 0835 or email epienaar@mrc.ac.za should you have any questions.

Yours faithfully,

Elizabeth D Pienaar
www.pactr.org Project Manager
+27 021 938 0835

THE SOUTH AFRICAN MEDICAL RESEARCH COUNCIL
Cochrane South Africa | PO Box 19070, Tygerberg, 7505
Tel: +27 (0)21 938 0438 | Email: cochrane@mrc.co.za | Web: www.southafrica.cochrane.org

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COMBINED PRODUCT/PROFESSIONALINDEMINTY INSURANCE POLICY

(CLINICAL TRIALS)

Head Office:
Aiico Plaza, Churchgate Street
Victoria Island. P. O. Box 2577. Lagos
Telephone: 2612527. 2612472. 2614669. 2610651.
E-mail: info@aiicoplc.com
Website: http://www.aiicoplc.com

AIICO INSURANCE
AMERICAN INTERNATIONAL

AIICO INSURACE PLC
PLOT 12 PC CHURCHGATE STREET
VICTORIA ISLALAGOS NIGERIA

THE SCHEDULE

POLICY NO:	**310/00001/17/TQ/HO**
INSURED:	DR. RICHARD ONALO
OCCUPATION /: PROFFESSION	**MEDICAL PRACTITIONER**
NATURE OF BUSINESS:	CLINICAL PRACTICE
ADDRESS:	DEPT. OF PAEDIATRICS, UNIVERSITY OF ABUJA TEACHING HOSPITAL, GWAGWALADA, ABUJA
COVERAGE:	**CLINICAL TRIALS.**
RISK DETAILS:	1. The proposer conducts clinical cardiovascular evaluation, including Ultrasonography examination. 2. The patients will also have L-arginine monohydrochloride orally
TRIAL CENTER	DEPT. OF PAEDIATRICS, UNIVERSITY OF ABUJA TEACHING HOSPITAL, GWAGWALADA, ABUJA
PREMIUM DUE:	**N50,000.00**
PERIOD OF INSURANCE:	FROM: 18TH SEPTEMBER, 2017 TO 17TH SEPTEMBER, 2018
RETROACTIVE DATE:	18TH SEPTEMBER, 2018
LIMITS OF INDEMNITY:	**N1, 000,000.00 / POLICY AGGREGATE** (INCLUSIVE OF CLAIMS EXPENSES)
DEDUCTIBLE:	**N50,000.00 OR 10% WHICHEVER IS HIGHER.**

BROKER : AMADI CHIKAODI AKWUGO-968/02/322

DATED IN LAGOS THIS 18TH DAY OF SEPTEMBER, 2017

.....
AUTHORISED SIGNATURE

1

APPENDIX 2.1: CASE REPORT FORM 1

M160326 PATIENT'S ID_____/____RANDOMIZATION No:_____ DRUG ARM___

CARDIAC CHANGES IN SICKLE-CELL CRISIS & ARGININE SUPPLEMENTATION

Consent process

Consent: Version and date of ICF: _____Date signed: _____ Explained by _____
Consent signed prior to any study-related procedure being performed: [ ]
Are you satisfied that the patient understands the consent form? [ ]Yes [ ]No
Did the patient ask anything about the study? [ ]Yes [ ]No
If yes, describe briefly:
Confirm Parent information sheet given to caregiver [ ]

Inclusion Criteria

All the following criteria are to be fulfilled (answered YES) for inclusion of an individual in the study.

Signed informed consent (+ assent) form [ ] Yes [ ] No
Child is having sickle cell crisis [ ] yes [ ] No
Child is at least 5years old [ ] Yes [ ] No
Child's pain score is at least 7 on a scale of 1-10 [ ] Yes [ ] No

Exclusion Criteria

Patient presenting with any of the following will not be included in the study

Child has acquired heart disease(s) [ ] Yes [ ] No
Child has need for immediate red cell transfusion [ ] Yes [ ] No
Child is receiving arginine contain product [ ] Yes [ ] No
Child allergic to L- Arginine? [ ] Yes [ ] No

SOCIAL DEMOGRAPHICS

Date of Birth: _____ Gender : [ ] Male [ ] Female

Parents level of education and occupational status: (Tick as appropriate)

Level of education	Father	Mother	Occupational status	Father	Mother
No formal education			Professional/ Senior Civil Servant		
Primary			Junior civil Servant		
Secondary			Skilled worker/ Businessman		
Post-secondary			Unskilled worker/Farmer		
University			Unemployed		

Parent's Socioeconomic Class (Oyedeji's Classification): (a) 1 (b) 2 (c) 3 (d) 4 and (e) 5

M160326 PATIENT'S ID_____/___RANDOMIZATION No:_____ DRUG ARM___

STAGE OF STUDY (Please tick one) Today's date_____

Pre–Arginine [ ] 6-days post Arginine [ ] 6 weeks post Arginine [ ]

Primary Clinical Diagnosis _____HB Concn _____Pain Score_____

Anthropometry

Parameters	Weight	Height	Wt-for-Ht	BMI	BSA	US	LS	AS
Measurements								
Z-Score								

CARDIAC PARAMETERS:

Pulse rate___bpm: SBP___ mmHg SBP% ___DBP___mmHg DBP% ___Mean BP___mmHg SPO_2__%

ECG Parameter	Duration	Amplitude	Interpretation	Intervals	Dimension	Interpretation
P-wave				PR		
QRS complex				QT		
T-wave				QTC		

Axis: P_____QRS_____T_____ QRS-T ANGLE____Depressed ST (leads___)(b) Elevated ST(leads_)

Transvalvular Regurgitations:

Site of regurgitation	Velocity (m/s)	Gradient	Site of regurgitation	Velocity (m/s)	Gradient
Aortic regurgitation			Tricuspid regurgitation		
Mitral regurgitation			Pulmonary regurgitation		

Estimated Pressures (mmHg)

RAP_____LAP_____RVSP_____PASP_____PADP_____MPAP_____

M-Mode ventricular dimensions

Structures (mm)	Dimensions	Z-score	Structures (mm)	Dimensions	Z-score
LVIDd			LVPWTd		
LVIDs			LVPWTs		
IVSd			EF (%)		
IVSs			FS (%)		

Index of Myocardial performance

LV systolic time_____ _ LVOT ET_____ LVTei_____ MAPSE_____, MAPSEZ___

RV systolic time_____ RVOT ET_____RV Tei_____ TAPSE_____, TAPSEZ___

Others: (a) Mitral E____ A_____ E/A_____Lateral TDI S'_____E'_____ A'_____ E/E'____IVA___

(b) RVOT VTI (cm) _____PVR_____PCWP_____Wall Stress Index _____ WSIZ___

Biochemical values: Arginine _____Ornithine_____Citrulline_____Troponin I____proBNP_____

M160326 PATIENT'S ID_____/____RANDOMIZATION No:_____ DRUG ARM___

UNIT DOSE DISPENSING OF SUPPLEMENT

Patient's weight_____ Kg Supplement arm code_____Actual supplement dose at 100mg/kg _____

Supplement dispensing @ 8 hourly schedule							
Day on supplement	Date Dispensed	Time Dispensed 1st dose	Dispensing officer's ID	Time Dispensed 2nd dose	Dispensing officer's ID	Time Dispensed 3rd dose	Dispensing officer's ID
Day 1							
Day 2							
Day 3							
Day 4							
Day 5							

ADVERSE EVENT LOG (Inquired before each dose of supplement)

Adverse event	Start date	Stop date	Intensity	Frequency	Relationship to Supplement	SAE
			Mild Moderate Severe	Once Intermittent Continuous	Not related Possibly related Probably related Definitely related	NO YES
			Mild Moderate Severe	Once Intermittent Continuous	Not related Possibly related Probably related Definitely related	NO YES
			Mild Moderate Severe	Once Intermittent Continuous	Not related Possibly related Probably related Definitely related	NO YES

CONCOMITANT MEDICATION LOG (To be obtained from patient's treatment sheet)

Concomitant Medications	Start date	Stop date	Dose	Route of administration	Frequency	Indication

APPENDIX 2.2: CASE REPORT FORM 2

M160326 PATIENT'S ID_____/____RANDOMIZATION No:_____ DRUG ARM___

ARGENTINE AS ADJUVANT THERAPY IN THE MANAGEMENT OF VOC PAINS

Consent process

Consent: Version and date of ICF: _____Date signed: _____ Explained by _____
Consent signed prior to any study-related procedure being performed: [ ]
Are you satisfied that the patient understands the consent form? [ ]Yes [ ]No
Did the patient ask anything about the study? [ ]Yes [ ]No
If yes, describe briefly:
Confirm Parent information sheet given to caregiver [ ]

Inclusion Criteria

All the following criteria are to be fulfilled (answered YES) for inclusion of an individual in the study

Signed informed consent (+ assent) form [ ] Yes [ ] No
Child is having vaso-occlussive crisis [ ] yes [ ] No
Child is at least 5years old [ ] Yes [ ] No
Child's pain score is at least 7 on a scale of 1-10 [ ] Yes [ ] No

Exclusion Criteria

Patient presenting with any of the following will not be included in the study

Child is having non-VOC pain [ ]Yes [ ] No
Child has fracture [ ] Yes [ ] No
Child has deep vein thrombosis [ ] Yes [ ] No
Clinical findings suggestive of osteomyelitis [ ] Yes [ ] No
Child has Neurologic Impairment (vision, hearing, or motor dysfunction [ ] Yes [ ] No
Child is allergic to arginine? [ ] Yes [ ] No

SOCIAL DEMOGRAPHICS

Date of Birth: _____ Gender : [ ] Male [ ] Female

Parents level of education and occupational status: (Tick as appropriate)

Level of education	Father	Mother	Occupational status	Father	Mother
No formal education			Professional/ Senior Civil Servant		
Primary			Junior civil Servant		
Secondary			Skilled worker/ Businessman/woman		
Post-secondary			Unskilled worker/Farmer		
University			Unemployed		

Parent's Socioeconomic Class (Oyedeji's Classification) : (a) 1 (b) 2 (c) 3 (d) 4 and (e) 5

Anthropometry

Parameters	Weight	Height	Wt-for-Ht	BMI	BSA	US	LS	AS
Measurements								
Z-Score								

M160326 PATIENT'S ID_____/_____RANDOMIZATION No:_____ DRUG ARM___

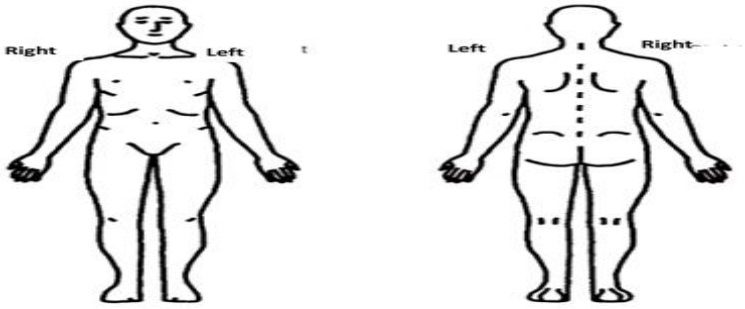
Day 1 Least Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain Worst possible pain

Pleas mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

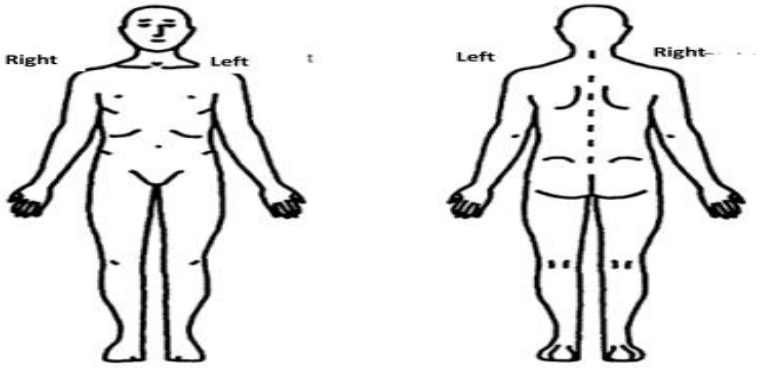
Day 1 Worst Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain Worst possible pain

Pleas mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

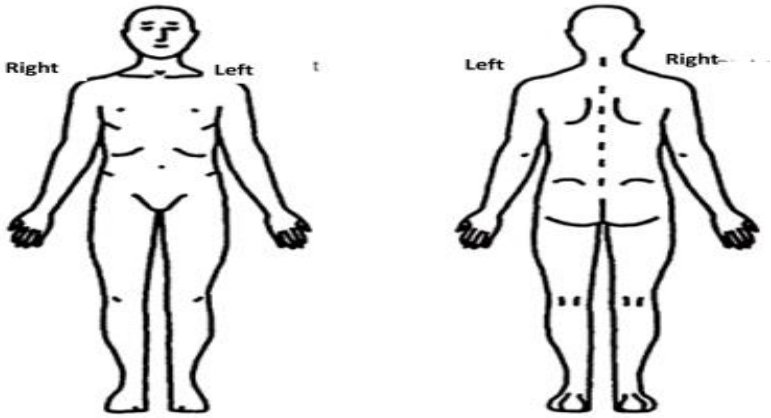
Day 1 Current Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain Worst possible pain

Pleas mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ]Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ]Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips[ ]Femur[ ] Knee [ ]
Tibia/Fibula[ ]Ankles [ ]

M160326 PATIENT'S ID_____/___RANDOMIZATION No:_____ DRUG ARM___

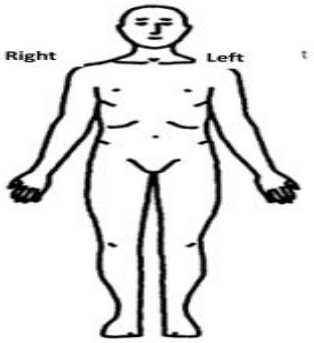
Day 2 Least Pain

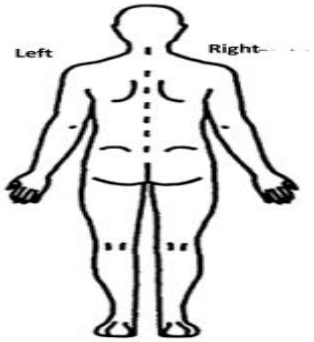
Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain Worst possible pain

Please mark an X where you hurt most





Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

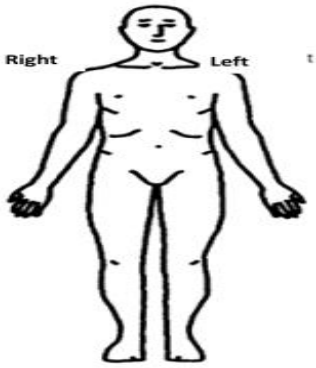
Day 2 Worst Pain

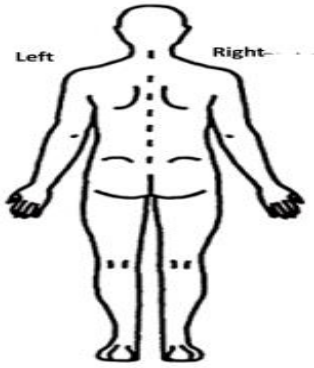
Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain Worst possible pain

Please mark an X where you hurt most





Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

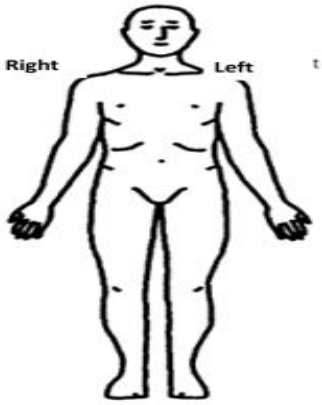
Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

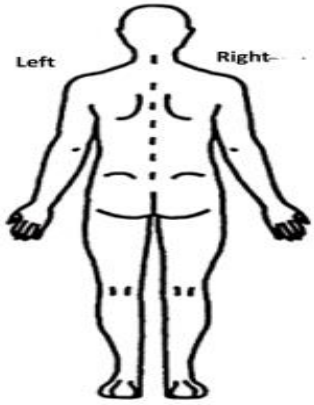
Day 2 Current Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain Worst possible pain





Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ]Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ]Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips[ ]Femur[ ] Knee [ ]
Tibia/Fibula[ ]Ankles [ ]

M160326 PATIENT'S ID__ ___/_____RANDOMIZATION No:____ DRUG ARM___

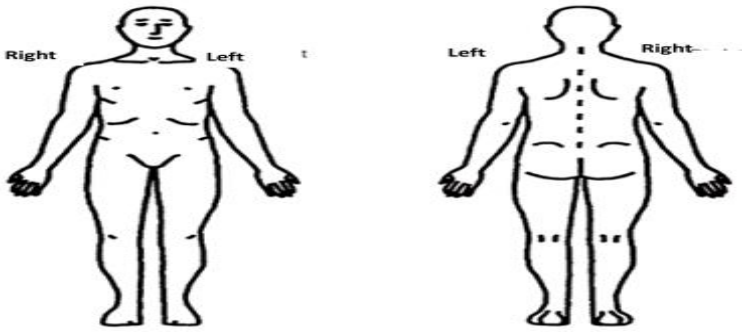
Day 3 Least Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain ——————————— Worst possible pain

Please mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

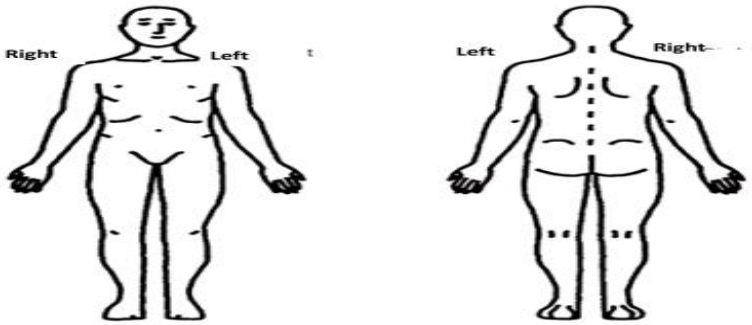
Day 3 Worst Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain ——————————— Worst possible pain

Please mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
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Lower Limb

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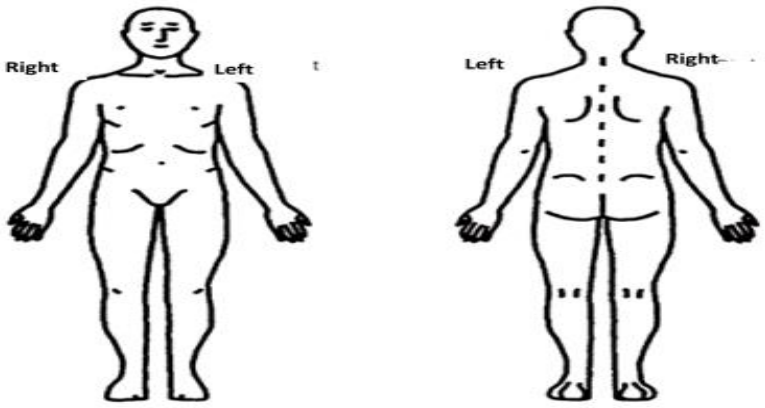
Day 3 Current Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain ——————————— Worst possible pain

Please mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ]Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ]Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips[ ]Femur[ ] Knee [ ]
Tibia/Fibula[ ]Ankles [ ]

M160326 PATIENT'S ID____/_____RANDOMIZATION No_____ DRUG ARM___

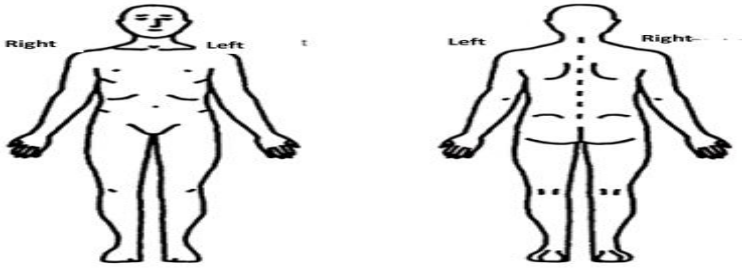
Day 4 Least Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain ——— Worst possible pain

Please mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

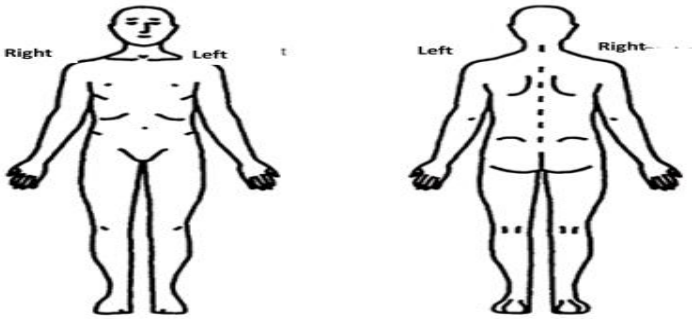
Day 4 Worst Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain ——— Worst possible pain

Pleas mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
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Lower Limb

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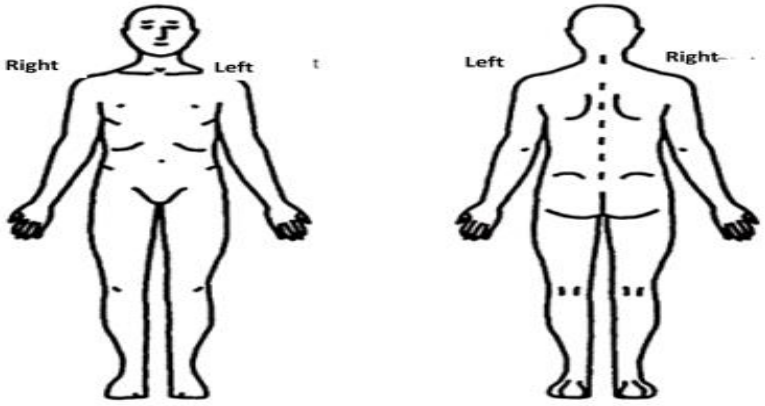
Day 4 Current Pain

Please circle the number that best describes your pain level

0 1 2 3 4 5 6 7 8 9 10

No pain ——— Worst possible pain

Pleas mark an X where you hurt most



Pease tick pain site

Axial Skeleton **Upper Lumbar**

Thoracic spine [ ]Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ]Humerus [ ]
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Wrist [ ]

Lower Limb

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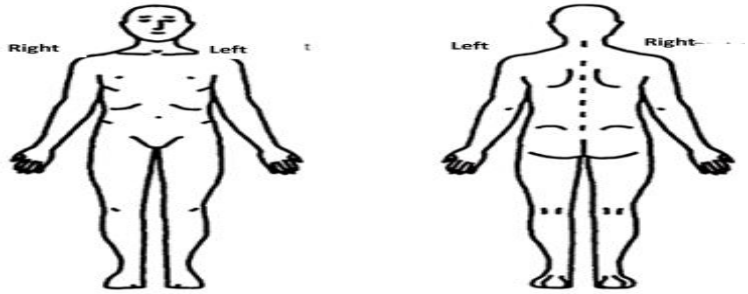
M160326 PATIENT'S ID_____/_____RANDOMIZATION No:_____ DRUG ARM___

Day 5 Least Pain

Please circle the number that best describes your pain level

No pain 0 1 2 3 4 5 6 7 8 9 10 Worst possible pain

Please mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

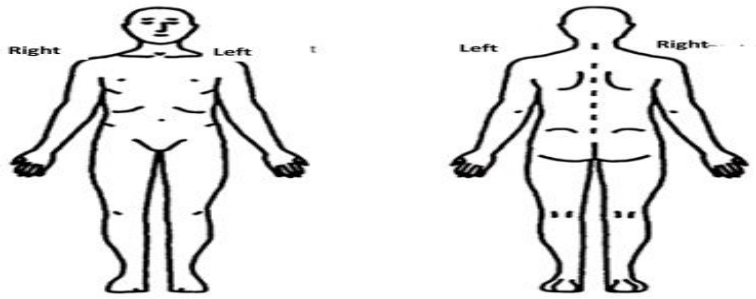
Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

Day 5 Worst Pain

Please circle the number that best describes your pain level

No pain 0 1 2 3 4 5 6 7 8 9 10 Worst possible pain

Please mark an X where you hurt most



Pease tick pain site

Axial Skeleton ***Upper Lumbar***

Thoracic spine [ ] Cavicles [ ]
Lumbar spine [ ] Shoulders [ ]
Abdomen [ ] Humerus [ ]
Sternum [ ] Elbows
Ribs [ ] Raidius/Ulna [ ]
Wrist [ ]

Lower Limb

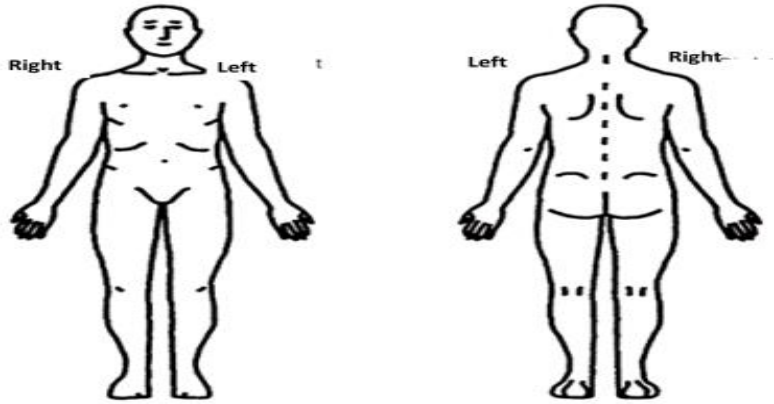
Hips [ ] Femur [ ] Knee [ ]
Tibia/Fibula [ ] Ankles [ ]

Day 5 Current Pain

Please circle the number that best describes your pain level

No pain 0 1 2 3 4 5 6 7 8 9 10 Worst possible pain

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Tibia/Fibula [ ] Ankles [ ]

APPENDIX 3: PDF of manuscript: Cardiovascular changes in children with sickle cell crisis

Cardiology in the Young

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Cardiovascular changes in children with sickle cell crisis

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Original Article

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Heart changes; pulmonary changes; sickle cell crisis; Nigerian children

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Abstract

Background: Sickle cell anaemia is characterised by frequent, sometimes serious events referred to as "crisis". Cardiopulmonary consequences such as pulmonary hypertension and myocardial ischaemia may accompany a serious crisis. *Objective:* To determine the cardiovascular changes that occur during a severe sickle cell crisis. *Methods:* A cross-sectional comparative study of sickle cell anaemia in children (5–17 years) admitted during a severe crisis (cases) and those in steady state (controls) was conducted over a 2-year period. Effects of the crisis on the cardiopulmonary system were assessed. The diagnosis of myocardial ischaemia was made using electrocardiography and serological cardiac biomarkers, while cardiac dysfunction and the presence of pulmonary hypertension were determined using echocardiography. The presence of systemic hypertension and tachycardia was also evaluated. *Results:* A total of 176 patients were recruited, 92 in steady state (male:female ratio, 1.2:1) and 84 in severe crisis (male:female ratio, 1.3:1). The mean age was 10.4 ± 3.2 years for steady state and 10.5 ± 3.4 years for those in crisis. The mean heart rate in crisis was higher than in steady state ($p < 0.0001$). The blood pressures (systolic, $p < 0.0001$, diastolic, $p < 0.0001$, mean, $p < 0.0001$) as well as myocardial ischaemia scores ($p < 0.0001$) were higher in patients with crisis than in those in steady state. Similarly, conduction abnormalities, pulmonary hypertension, and ventricular dysfunction were more prevalent in the crisis than in the steady state. *Conclusion:* The present data suggest that sickle cell crisis results in a derangement of clinical, electrocardiographical, and echocardiographical parameters in children with sickle cell anaemia. Further research on these cardiovascular events may improve the overall care of these patients.

Sickle cell anaemia, a common chronic debilitating haematological disease,[1] is prevalent in sub-Saharan Africa[2] with Nigeria having the largest number of children with the condition world wide.[3] It is one of the highest non-infectious cardiopulmonary causes of mortality in children.[4–6]

Most data describing the cardiovascular complications in sickle cell anaemia come from the adult literature where only steady-state changes were obtained.[7–11]

Pain which is the clinical hallmark of vaso-occlusive crisis is debilitating for the sufferer and is associated with high hospital admission rates, a high economic burden, and loss of school hours. Severe painful crisis is a major cause of morbidity[12,13] and an important contributor to mortality in adults and children with sickle cell anaemia. Acute painful crises are triggers for the acute chest syndrome,[5,6,14] which is one of the leading causes of morbidity and the most common cause of mortality in sickle cell anaemia patients.[4,5] Both vaso-occlusive crisis and acute chest syndrome cause acute increases in pulmonary artery pressure[7,8] which can be measured indirectly using echocardiography. Even a mild but acute increase in pulmonary artery pressure could be devastating and may result in cardiovascular collapse and sudden death. Studies[9,10] in adult patients with sickle cell anaemia have shown that the tricuspid regurgitation velocity of ≥2.5 m/s using echocardiography increases the risk of death 10-fold and is a major independent risk factor for sudden death.[9] In addition, electrocardiographic evidence of ischaemic cardiac changes that were thought to be rare in children are now being frequently documented during vaso-occlusive crisis and acute chest syndrome thus suggesting that these conditions might have been under-diagnosed and under-reported.[15,16] It is therefore necessary to evaluate the effects of severe sickle cell crisis on the cardiovascular system in order to establish the prevalence of these conditions in children with sickle cell crisis and provide a platform for advocating anticipatory care during episodes of severe sickle cell crisis in at-risk patients.

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Methodology

Study location

This study was conducted in the Federal Capital Territory of Nigeria, Abuja. There are two well-established tertiary health facilities in the area, namely the National Hospital Abuja and the University of Abuja Teaching Hospital.

Paediatric sickle cell clinics are held weekly in these hospitals. A total of 800 sickle cell disease patients are registered on the clinics database. The majority of the patients (95%) have sickle cell anaemia. Children who develop mild vaso-occlusive crisis are treated in the clinic, while those with severe pain and other manifestations of crisis are hospitalised in the emergency unit in the respective hospitals.

Study design

A cross-sectional comparative study involving age- and sex-matched children with sickle cell anaemia between ages 5 and 17 years with any form of severe crisis (cases) warranting hospital admission and those in steady state (controls) was conducted. Children in steady state (haemoglobin SS patients with no acute illness in the preceding 4–6 weeks) were obtained from the weekly held sickle cell clinic. The cases with sickle cell crisis were obtained from the Emergency Paediatric Unit.

Exclusion criteria

Children with concomitant heart diseases and metabolic conditions were excluded.

Study outcome variables

Primary outcome was the proportion of children with myocardial ischaemia determined by electrocardiography and a serological cardiac biomarker (N-terminal pro B-type natriuretic peptide ≥160 pg/ml, cardiac troponin I > 0.2 ng/ml).

Secondary outcomes determined using echocardiography included (a) systolic and diastolic cardiac dysfunction using Doppler parameters, (b) tricuspid regurgitation velocity >2.5 m/s and Doppler-estimated mean pulmonary artery pressure >25 mmHg, and clinical parameters such as systemic blood pressure and heart rates

Sample size calculation was based on the data published by Bode-Thomas et al.[15] from Jos, north-central Nigeria on the prevalence of significant myocardial ischaemia in children with vaso-occlusive crisis (89.5%) and those in steady state (72.5%). Using Stata 14 statistical software, a power of 80%, a significance level of 5%, a sample size of 166 patients (83 patients in steady state and 83 patients in crisis) were shown to be sufficient in order to detect a significant influence of sickle cell crisis on the prevalence of myocardial ischaemia. For the study, 176 patients were recruited, comprising 92 patients in steady state and 84 patients in crisis.

Study procedure

Children that fulfilled the inclusion criteria were recruited by convenience (non-probability) sampling. The patients had a single pulse oximetry, blood pressure, and heart rate measurements performed manually, as well as a single standard 12-lead surface electrocardiography and a single transthoracic echocardiography. Blood samples were taken for cardiac biomarkers for the two groups of patients (in the haematology clinic for those in steady state and during admission for those in crisis. Patients in whom significant cardiac abnormalities such as systemic blood pressures >99 percentile + 5 mmHg (eight patients), tricuspid regurgitation ≥3 m/s (five patients), and elevation of mean pulmonary pressure >40 mmHg (three patients) were referred to the cardiac clinic for follow up but their follow-up details are not included in this report.

Detailed study procedures

The processes involved in the study procedures were explained to each parent/child, with a demonstration to reassure them of its safety and to calm the child. For those recruited during a crisis episode, the measurements were taken after administration of pain medications, once the pain had subsided and the patient was calm.

Blood pressure measurement

A supine brachial blood pressure was obtained manually with an appropriate size cuff of the mercury sphygmomanometer (AccosonDekamet®; A.C. Cossor and Son Surgical Ltd, Accoson Works, Harlow, Essex, UK) applied to the right arm. The results were interpreted according to normative referenced values[17]

Electrocardiographic evaluation

A conventional 12-lead resting electrocardiography (using Biocare 12Ch Digital Resting ECG W/5.7" LCD Foldable Screen, ECG-1210) was then recorded with the child lying on a couch in supine position, according to a standardised protocol.[18] A minimum of five cardiac cycles were recorded per lead. All measurements were done manually with the aid of a magnifying lens according to standard guidelines. The values obtained were interpreted using both Nigerian[19] and other reference values used for Caucasian children.[20] Various electrocardiographic parameters were used to compute the myocardial ischaemia score as described by Bode-Thomas et al.[15] (Appendix in the Supplementary Material). These parameters included prolonged PR and corrected QT intervals, >98th percentile for age and sex, as well as ST-T abnormalities (flat and/or inverted T-wave and ST elevation and depression) as defined by Park and Guntheroth.[21]

Echocardiographic evaluation

Standard two-dimensional, M-mode, and Doppler echocardiograms were performed by the researcher in the supine and left lateral decubitus positions and measurements done according to the American Society of Echocardiography guidelines.[22] A General Electric Vivid-e echocardiography machine with a high-resolution 5–7.5 MHz sequential transducer and an integrated calculation package was used. All imaging was captured with a simultaneous 3-lead electrocardiographic tracing. Standard parasternal, apical, subcostal, and suprasternal views were recorded. Left ventricular end-diastolic and end-systolic dimensions, septal and left ventricular posterior wall thickness at end diastole and end systole, mitral inflow velocities, left and right ventricular outflow tract velocity time integral (VTI_{LVOT}, VTI_{RVOT}), tricuspid and pulmonary valves regurgitation jet velocities (TRV, PRV) were captured. Left ventricular ejection fraction, fractional shortening, and cardiac output were computed by the machine, while pulmonary artery systolic (PASP) and diastolic (PADP) pressures were estimated from the peak tricuspid and pulmonary regurgitation velocities, respectively, using the simplified Bernoulli equation ($P = 4V^2$, where P = pressure, V = peak velocity of regurgitant jet) and the right atrial pressure (obtained from inferior vena cava dimensions and collapsibility index). Mean pulmonary artery pressure

(mPAP) was calculated from the formula: mPAP = 2/3 PADP + 1/3 PASP, while pulmonary vascular resistance (PVR)[23] and systemic vascular resistance (SVR)[24] were obtained using the equation: PVR = 10 (TRV/VTI_{RVOT}) + 0.16; SVR = Mean Arterial Pressure/Cardiac Index.

The velocity of circumferential fibre shortening and end-systolic wall stress (meridional and circumferential) was calculated using published formulas[25] (Appendix in the Supplementary Material). Pulse Wave Doppler Tei index of the left and right ventricles was determined from the ratio of the summation of isovolemic relaxation and contraction times to the ejection time. Mitral and tricuspid annular plane systolic excursion were measured using the standard M-mode technique with the cursor placed at the lateral site of the annulus from the apical four-chamber view and interpreted using published normative values[26,27] All measurements were made online by a single investigator using an average of three cardiac cycles. The images were saved and re-analysed offline by the same researcher to obtain a second measurement.

Serum biochemical analysis

A single sample 5 ml of venous blood was collected into a lithium heparin bottle from the antecubital vein of each patient. The blood samples were centrifuged at 3500 rpm for 15 minutes by the researcher or trained assistant. Specimens for plasma NT-pro BNP and cardiac troponin I were stored at −80°C until analysed using an electrochemiluminescence immunoassay.

Statistical analysis

Mean values of heart rates, blood pressures, and oxygen saturation during crisis and steady states were compared using a paired t-test for variables with normal distribution, while the Shapiro–Wilk test was used for variables not normally distributed. The relationship between myocardial ischaemia score and the variables of pulmonary and systemic circulations was tested using Pearson's correlation matrix. The chi square test was used to test the differences between categorical variables. Intraobserver reproducibility of echocardiographic measurements was tested according to the method of Bland and Altman. All analyses were performed with Stata 14 software. A p value of less than 0.05 was set as level of statistical significance.

Ethical approval

The study was approved by the Human Research Ethics Committee of University of the Witwatersrand, Johannesburg, South Africa and the Health Research Ethics Committees of University of Abuja and National Hospital, Abuja, Nigeria.

Results

General characteristics

A total of 176 children were recruited, comprising of 92 steady-state patients and 84 patients in crisis. The age at presentation was similar for both groups: 10.4 ± 3.2 years for steady state and 10.5 ± 3.4 years for those in crisis, p = 1.00. The male:female ratio was 1.2:1 for steady state and 1.3:1 for those in crisis. The average steady-state haemoglobin concentration was 7.9 g/dl and 7.7 g/dl, respectively, for patients recruited without crisis and those recruited at the time of crisis, p = 0.98. Similarly, the mean lifetime blood transfusion was comparable in both groups: 1.2 ± 1.7 for patients in steady state and 1.3 ± 1.4 for those in crisis, p = 0.67, while the mean number of blood transfusion in the preceding 1 year was 0.5 + 0.7 for patients in steady state and 0.5 ± 0.5 for those enrolled during crisis.

Reproducibility assessment

Table 1 shows the results of intraobserver variability in cardiac parameter assessment. The echocardiographic measurements showed minor intraobserver difference between the online and off-line measurements. The mean difference in the tricuspid regurgitation velocities, for instance, was 0.034 (CI −0.04 to 0.104) (r = 0.061, p = 0.798) with a 95% limits of agreement of −0.282 to 0.350.

Heart rate and blood pressure changes in crisis

The mean heart rate in patients with crisis (98.1 ± 14.2 bpm) was higher than in those in steady state (86.9 ± 1.4 bpm) (p < 0.01). The blood pressures (systolic, p < 0.01, diastolic, p < 0.01, mean, p < 0.01) were also higher in the crisis group than in the steady-state group (Fig 1). During crisis, 40.2% had a systolic blood pressure greater than the 95th percentile (21.6% of those were higher than the 99 + 5 mmHg percentile). In the steady-state group, markedly elevated blood pressures (>99 + 5 mmHg percentile) were not documented. Only 5.4% had a systolic blood pressure greater than the 95th percentile.

Similarly, elevations in the diastolic blood pressure were observed, with 31% of patients recorded to have a diastolic blood pressure greater than the 95th percentile (19.2% of those were greater than the 99 + 5 mmHg percentile) during crisis, while only 9.6% of those in steady state had blood pressure in excess of the 95th percentile. None were greater than the 99th percentile.

Prevalence of conduction abnormalities

The prevalence of prolonged PR interval (5.4%) and prolonged corrected QT interval (32.6%) during the steady state was about half the prevalence in crisis (13.3% for prolonged PR interval and 55.4% for prolonged corrected QT interval), Figure 2. Sickle cell crisis doubled the prevalence of interventricular conduction delay from 7.6% in the steady-state group to 14.5% in the crisis group.

The myocardial ischaemia score (a composite of T-wave abnormalities, ST segment changes, and conduction abnormalities) had a negative correlation with haemoglobin concentration at presentation (r = −0.21, p = 0.01), oxygen saturation (r = −0.30, p < 0.01), and systemic vascular resistance (r = −0.30, p < 0.01), but correlated positively with number of blood transfusion in the preceding year, pulmonary artery pressures, right ventricular Tei index, and left ventricular wall thickness as shown in Table 2. The mean myocardial ischaemia score obtained in patients having crisis (3.14 ± 1.61) was higher than that recorded in patients recruited at steady state (1.97 ± 1.41), p < 0.01. Likewise, those with acute chest syndrome (n = 11) had higher mean ischaemia score than those without acute chest syndrome: 4.4 ± 1.59 versus 3.33 ± 1.28, p = 0.02.The mean N-terminal pro B-type natriuretic peptide was also higher in those with acute chest syndrome than in those without acute chest syndrome: 980.46 ± 1498.92 pg/ml versus 175.30 ± 664.00 pg/ml, p = 0.01.

Echocardiographic changes detected during crisis in the right and left heart

Both tricuspid and pulmonary regurgitation velocities as well as pulmonary artery pressures and pulmonary vascular resistance calculations were elevated during crises. Similarly, the mean circumferential

Table 1. Intraobserver reproducibility of cardiac parameters measurements.

Cardiac Parameters	Mean difference	95% CI of mean difference	ρ	p	95% limits of agreement
Tricuspid regurgitation velocity	0.034	−0.04, 0.108	−0.061	0.788	−0.282, 0.350
Pulmonary regurgitation velocity	0.071	−0.144, 0.002	0.088	0.711	−0.384, 0.242
Left ventricular internal dimension in diastole	0.062	−0.126, 0.249	−0.102	0.670	−0.739, 0.862
Left ventricular internal dimension in systole	0.018	−0.206, 0.170	−0.073	0.761	0.821, 0.785
Interventricular septum in diastole	−0.006	−0.093, 0.082	0.454	0.044	−0.378, 0.367
Interventricular septum in systole	0.058	−0.015, 0.131	0.046	0.848	−0.253, 0.369
Left ventricular posterior wall thickness in diastole	−0.002	−0.059, 0.054	0.428	0.060	−0.243, 0.238
Left ventricular posterior wall thickness in systole	0.116	−0.010, 0.223	0.220	0.351	−0.340, 0.573
Mitral annular plane systolic excursion	0.042	−0.124, 0.040	−0.007	0.977	−0.390, 0.306
Tricuspid annular plane systolic excursion	0.027	−0.079, 0.094	−0.090	0.705	−0.363, 0.378
Left ventricular ejection fraction	−0.100	−1.315, 1.116	−0.308	0.186	−5.292, 5.093
Left ventricular fractional shortening	0.195	−1.941, 1.321	−0.002	0.387	−2.990, 3.380

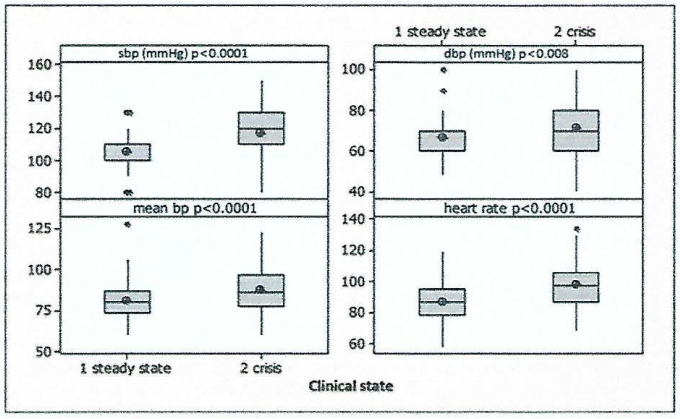


Figure 1. Boxplot of heart rates and blood pressures in sickle cell crisis and in steady state.
Key: sbp = systolic blood pressure, dbp = diastolic blood pressure, mean bp = mean blood pressure. Boxplot explanation: upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; horizontal bar within box, median; asterisk within and outside the box, mean and outlier, respectively.

wall stress index in patients having crisis (218.0 ± 59.5 g/cm^2) was higher than the value recorded for steady-state patients (195.1 ± 44.9 g/cm^2), $p = 0.01$, but there was no statistically significant difference in the meridional wall stress index in the two groups: 79.2 ± 23.4 g/cm^2 (*steady state*) *versus* 88.5 ± 30.8 g/cm^2 (*crisis* group), $p = 0.05$ (Table 3). The left ventricular fractional shortening ($35.7 \pm 5.0\%$) and ejection fraction ($64.8 \pm 6.8\%$) mean values in steady state were comparable to those obtained in crisis state: $37.1 \pm 5.3\%$ and $66.6 \pm 7.0\%$, respectively.

The interval plot of mean of tricuspid regurgitation, pulmonary artery systolic and diastolic pressures shows a significant difference between values documented in the steady state and those in crisis. No significant difference in the mean pulmonary regurgitation velocity was shown between the two groups (Fig 3).

Prevalence of ventricular dysfunction and pulmonary hypertension

The prevalence of left ventricular dysfunction (left ventricular Tei index >0.45) was 2.5-fold higher in patients having crisis than in patients without crisis: 16.7% versus 6.5%, $p = 0.03$ (Fig 4 and Table 4). Likewise, the number of children with mitral annular plane systolic excursion z score <−2 was five times higher in the crisis group than in the steady-state group: 13 (15.5 %) versus 3 (3.3%), $p = 0.01$.

The prevalence of right ventricular dysfunction (right ventricular Tei index value >0.4) was three times higher in the crisis group than in the steady-state group (3.3% in steady state versus 10.7% in crisis). This difference, however, fell short of statistical significance, $p = 0.05$. (Fig 4 and Table 4).

The prevalence of pulmonary hypertension (mean pulmonary artery pressure >25 mmHg) tripled when comparing those in steady state with those in crisis (14.1% versus 47.6%) (Table 4).

The interval plot of systemic vascular resistance, pulmonary vascular resistance, and wall stress index mean values are shown in Figure 5. The interaction test for systemic vascular resistance and meridional wall stress index shows a p value of > 0.05. The interaction test for pulmonary vascular resistance and circumferential wall stress index in steady state and crisis shows a $p < 0.01$ and 0.01, respectively.

Table 2. Correlation of myocardial ischemia score with clinical and echocardiographic parameters.

Parameters	r	p
Number of admission in the preceding year	0.14	0.08
Number of blood transfusion in the preceding year	**0.20**	**0.01**
Lifetime blood transfusion	**0.19**	**0.02**
Hemoglobin concentration at presentation	**−0.21**	**0.01**
Systolic blood pressure	**0.29**	**<0.01**
Diastolic blood pressure	0.13	0.06
Mean blood pressure	**0.20**	**<0.01**
Oxygen saturation	**−0.30**	**<0.01**
Corrected QT interval	**0.47**	**<0.01**
Tricuspid regurgitation velocity	**0.38**	**<0.01**
Pulmonary regurgitation velocity	**0.23**	**<0.01**
Pulmonary artery systolic pressure	**0.44**	**<0.01**
Pulmonary artery diastolic pressure	**0.35**	**<0.01**
Mean pulmonary artery pressure	**0.45**	**<0.01**
Left ventricular end-diastolic dimension z score	0.14	0.05
Left ventricular posterior wall thickness z score	**0.26**	**<0.01**
Mitral annular plane systolic excursion z score	**0.15**	**0.03**
Left ventricular Tei index	0.07	0.34
Right ventricular Tei index	**0.16**	**0.02**
Tricuspid annular plane systolic excursion z score	0.14	0.05
Pulmonary vascular resistance	**0.17**	**0.01**
Systemic vascular resistance	**−0.30**	**<0.01**

p value < 0.05 signifies statistical significance.

Discussion

Sickle cell crisis triggers a cascade of events in the cardiovascular system, including increases in heart rate, blood pressure, electrical and functional abnormalities. In this study, the heart rate was higher in those in crisis compared with those in steady state, by an average of 12 bpm, a finding corroborated by the report of other authors.[27] Many factors in crisis acting independently and/or cumulatively could increase the heart rate including pain, fever, hypoxia, and myocardial ischaemic injury. Tachycardia as a compensatory mechanism can cause an increase in oxygen consumption with a possible worsening of myocardial ischaemia.

Sickle cell patients generally have lower blood pressures than individuals with normal haemoglobin genotype (haemoglobin AA).[28,29] However, a transient elevation in systemic blood pressures has been documented in sickle cell crisis.[30] An average difference of 14 mmHg in systolic pressures was observed between children in crisis and those in steady state. Of those in crisis, 40.2% had systolic blood pressures greater than the 95th percentile, and 21.6% of those were higher than the 99 + 5 mmHg percentile. In contrast, only 5.4% of children in the steady state were documented to have systolic blood pressures greater than the 95th percentile and none were found to have blood pressures greater than the 99 + 5 mmHg percentile in the present study. Elevations in diastolic blood pressures were also observed, with 31% of those in crisis having diastolic blood pressures greater than the 95th percentile, and 19.2% of those were greater than the 99 + 5 mmHg percentile. A possible explanation for the elevation of blood pressure in sickle cell crisis is the marked increase in total blood volume and cardiac output during crisis.[31–34] Acute elevations of blood pressures in excess of the 99 + 5 mmHg percentile may alter the regulation of the blood–brain barrier resulting in cerebral hyperperfusion syndromes which manifest with symptoms such as headaches. Furthermore, children with very high blood pressures have been shown to be at-risk for

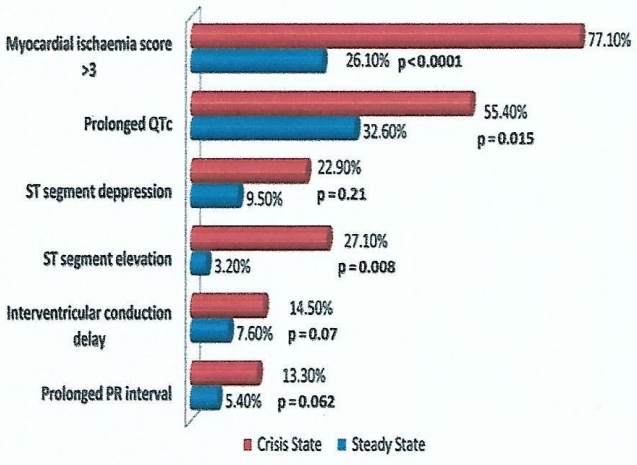


Figure 2. Influence of sickle cell crisis on the prevalence of prolonged PR and QTc, ST segment changes, and significant myocardial ischaemia scores. p value < 0.05 signifies statistical significance.

Table 3. Comparison between echocardiographic parameters of patients in steady state and of those in crisis.

Echocardiographic indices	Steady state	Crisis	p
Tricuspid regurgitation (m/s)	1.52 ± 0.84	2.17 ± 0.93	**<0.01**
Tricuspid regurgitation gradient (mmHg)	12.02 ± 8.49	22.26 ± 11.72	**<0.01**
Pulmonary regurgitation velocity (m/s)	1.05 ± 0.62	1.28 ± 0.77	0.09
Pulmonary regurgitation gradient (mmHg)	5.95 ± 5.06	8.73 ± 7.76	**0.01**
Pulmonary artery systolic pressure (mmHg)	22.71 ± 8.81	33.77 ± 12.12	**<0.01**
Pulmonary artery diastolic pressure (mmHg)	16.65 ± 5.29	20.62 ± 8.36	**<0.01**
Mean pulmonary artery pressure (mmHg)	18.67 ± 5.16	25.00 ± 7.78	**<0.01**
Pulmonary vascular resistance (wu)	0.92 ± 0.43	1.44 ± 1.44	**0.02**
Pulmonary capillary wedge pressure (mmHg)	8.45 ± 2.56	8.26 ± 1.44	1.00
Meridional wall stress index (g/cm^2)	79.2 ± 23.4	88.5 ± 30.8	0.05
Circumferential wall stress index (g/cm^2)	195.1 ± 44.9	218.0 ± 59.5	**0.01**
Systemic vascular resistance (Wu)	18.45 ± 5.36	18.13 ± 6.18	1.00
Left ventricular ejection fraction (%)	64.8 ± 6.8	66.6 ± 7.0	1.00
Left ventricular fractional shortening (%)	35.7 ± 5.0	37.1 ± 5.3	1.00
Ratio of right ventricular systolic pressure to systemic systolic pressure	022 ± 0.09	0.30 ± 0.10	<0.01

p value < 0.05 signifies statistical significance.

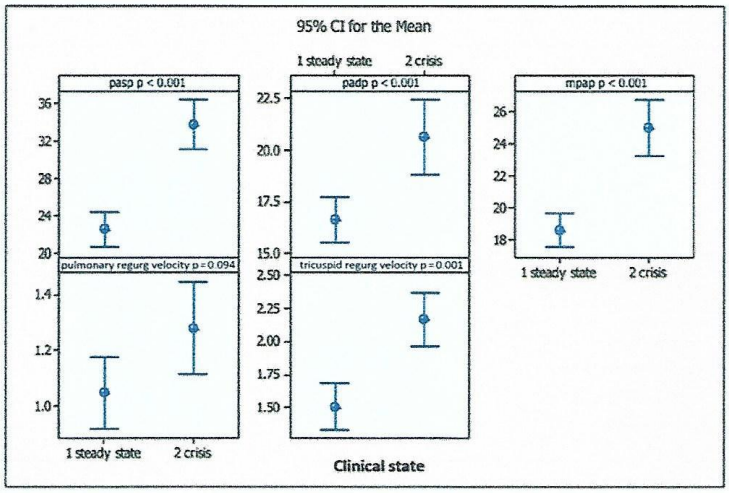


Figure 3. Interval plot of mean of indices of pulmonary hypertension in crisis and in steady-state patients.
Key: mpap = mean pulmonary artery pressure, pasp = pulmonary artery systolic pressure, padp = pulmonary artery diastolic pressure, regurg = regurgitation.

silent strokes and associated memory loss. The same report showed the relative risk of silent stroke in sickle cell disease children to be 1.7 times of those with lower blood pressures.[32] Although four patients with crisis in our study presented with overt stroke, the role of blood pressure elevation in their stroke could not be determined. An elevated systemic blood pressure during crisis may be associated with the development of left ventricular dysfunction.[35] Although there is no definitive proof, the elevated blood pressures recorded during crisis could have had a role in the twofold increase in the proportion of children with cardiac dysfunction compared to those in a steady state. Further studies are required to confirm this relationship.

Acute increases in pulmonary artery pressures recorded in the presence of severe vaso-occlusive bone pain crisis and acute chest syndromes[36] are poorly tolerated by adult sickle cell patients.[7] An increase of 10 mmHg in the mean pulmonary artery pressure has been associated with 1.7-times increase in the hazard ratio of death.[37] The acute pressure change exerts pressure overload on the right ventricle which could precipitate right ventricular dysfunction. The proportion of patients with high pulmonary artery pressures and right ventricular dysfunction was higher in the crisis than in the steady-state group in this study.

Electrocardiographic evaluations of patients with sickle cell crisis revealed features suggestive of myocardial ischaemia.[15,33]

Table 4. Comparison of the prevalence of cardiac dysfunction and pulmonary hypertension in steady state and in crisis.

Index of cardiac dysfunction and pulmonary hypertension	Number of patients, n (%)		
	Steady state	Crisis	p
Left ventricular ejection fraction <55%	6 (6.52)	8 (9.52)	0.46
Left ventricular fractional shortening <28%	6 (6.52)	4 (4.76)	0.61
Left ventricular Tei index >0.45	6 (6.52)	14 (16.67)	**0.03**
Mitral annular plane systolic excursion z score <–2	3 (3.26)	13 (15.48)	**0.01**
Right ventricular fractional area change <35%	14 (15.22)	16 (19.05)	0.50
Right ventricular Tei index >0.40	3 (3.26)	9 (10.71)	0.05
Tricuspid annular plane systolic excursion z score <–2	9 (9.78)	13 (15.48)	0.25
Tricuspid regurgitation velocity >2.5 m/s	6 (6.52)	43 (51.19)	**<0.01**
Tricuspid regurgitation gradient >30 mmHg	0	24 (51.19)	**<0.01**
Pulmonary artery systolic pressure >40 mmHg	1 (1.09)	30 (35.71)	**<0.01**
Mean pulmonary artery pressure >25 mmHg	13 (14.13)	40 (47.62)	**<0.01**
N-Terminal pro B-type natriuretic peptide >160 pg/ml	13 (14.13)	33 (38.82)	**<0.01**
Cardiac troponin I > 0.2 ng/ml	6 (6.52)	23 (27.07)	**<0.01**

p valve < 0.05 signifies statistical significance.

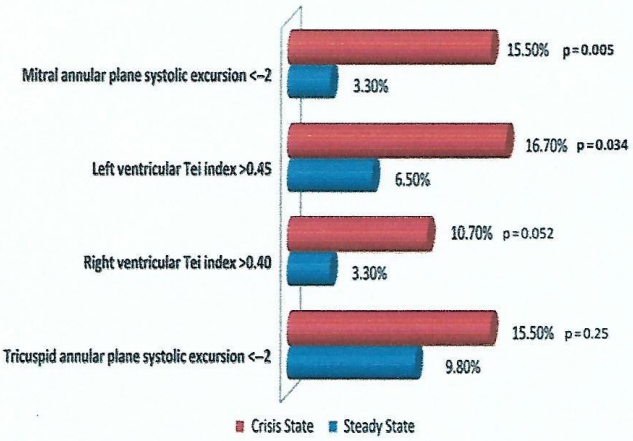


Figure 4. Prevalence of abnormal Tei index and annular plane systolic excursion in crisis and in steady state. p value < 0.05 signifies statistical significance.

In this study, ST segment abnormalities, particularly ST elevation, and prolonged PR and QT intervals were more prevalent during crisis. However, unlike Maisel et al.[34] who used 12–24 hour continuous electrocardiographic monitoring, the presence of ventricular arrhythmias in the study patients was not documented, most likely because a single 12-lead electrocardiogram was performed during admission for sickle cell anaemia crisis.

T-wave abnormalities suggestive of myocardial ischaemia (flat T-waves, T-wave inversion in more than two contiguous leads) have been documented in children with sickle cell crisis in a study from Nigeria by Bode-Thomas et al.[15] The use of the composite myocardial ischaemia score devised by these authors as well as the addition of cardiac biomarkers further increases the sensitivity and specificity of the diagnosis of myocardial ischaemia. The majority of children with sickle cell crisis in the present study (77.1%) fulfilled the Bode-Thomas' electrocardiographic criteria for myocardial ischaemia. However, the prevalence of myocardial ischaemia was significantly reduced to 40% when elevated myocardial injury biomarkers such as N-terminal pro B-type natriuretic peptides and cardiac troponin I were added as evidence of myocardial injury suggesting the need for caution when using ST-T wave abnormalities alone.

Radionuclide cardiac scans and autopsy results[16] have shown the mechanism of myocardial ischaemia to be due to microvascular occlusion with sickled red blood cells in some patients. The chest pain experienced by certain patients during crisis in the present study may be due to a vaso-occlusive process resulting in myocardial ischaemia. Further evidence of myocardial ischaemia caused

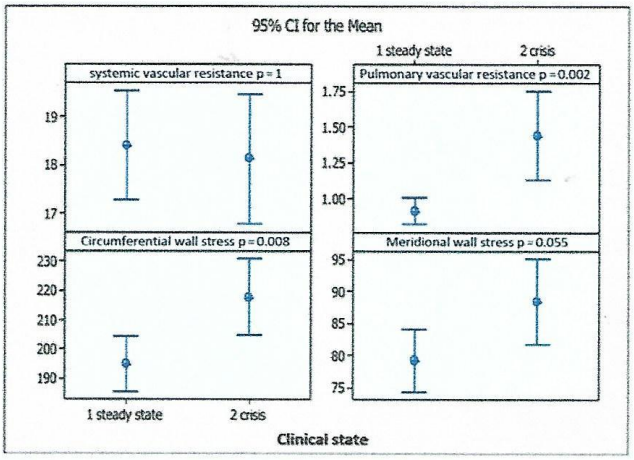


Figure 5. Plot of systemic vascular resistance, pulmonary vascular resistance and wall stress index in steady state and crisis.

by vascular occlusion is the finding of left ventricular wall motion abnormalities by Maisel et al.[34] during sickle cell crisis in patients presenting with an acute chest syndrome and severe vaso-occlusive bone pain crisis.

Echocardiographical imaging in the present study showed that the prevalence of left ventricular dysfunction (based on left ventricular Tei index > 0.45 and mitral annular plane systolic excursion z score < −2) was about three times higher in patients having crisis than in those in steady state. Furthermore, right ventricular dysfunction (right ventricular Tei index > 0.4) is more prevalent during a crisis than in the steady state.

The presence of elevated pulmonary artery pressures during a crisis is reflected by the tricuspid regurgitant jet velocity which was higher (mean of 2.2 m/s) in the crisis group than in the steady-state group (mean of 1.52 m/s). Numerous publications[7–10,14–16] have shown up to a fourfold increase in mortality and sudden death in patients with elevated pulmonary pressures during a crisis. In the present study, the mean pulmonary artery pressure in patients with sickle cell crisis was higher than those of patients in steady state with 47.6% of those in crisis having a mean pulmonary artery pressure greater than 25 mmHg compared to 14.1% in the steady-state group. One patient who presented with acute chest syndrome in the current study died suddenly during the crisis before a full echocardiographic and electrocardiographic evaluation could be done.

In this study, the authors have identified pertinent cardiovascular changes that may occur during sickle cell crisis to include elevation in blood pressures, subclinical myocardial ischaemia, reduction in systolic performance, and increases in severity of tricuspid regurgitation and hence pulmonary artery pressure.

Conclusions

The present data suggest that sickle cell crisis has negative effects on the cardiovascular system causing elevations in blood pressures, myocardial ischaemia, left ventricular dysfunction, and elevation of pulmonary artery pressures.

Recommendations

A repetition and follow-up of the clinical, biochemical, echocardiographic, and electrocardiographic parameters between crises may provide confirmation of whether outcomes such as sudden death are due to accumulative damage of the myocardium caused by repeated episodes of systemic hypertension versus pulmonary hypertensive events or vaso-occlusive coronary artery disease.

Limitations of study

- No follow-up of patients following crises.
- No facility to undertake direct intracardiac catheter measurements such as pulmonary artery pressure.
- Indirect assessment of pulmonary artery pressures and calculation of pulmonary vascular resistance and other cardiac indices using echocardiographic-acquired parameters.

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Conflict of interest. The authors declared that the research was conducted in the absence of any financial relationship that could be considered a potential source of conflict of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of Nigerian Code of Health Research Ethics on human experimentation and with the Helsinki Declaration of 1975 as revised in 2008 and have been approved by the Human Research Ethics Committee of University of the Witwatersrand, Johannesburg, South Africa and the Health Research Ethics Committees of University of Abuja Teaching Hospital and National Hospital, Abuja, Nigeria.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951120000037

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APPENDIX 4: PDF of short article published in the Blood journal: Oral arginine as adjuvant therapy

Oral Arginine Therapy As a Novel Adjuvant in the Management of Acute Pain in Children with Sickle Cell Anemia in Nigeria: A Randomized Placebo-Controlled Trial

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Introduction: Severe vaso-occlusive pain episodes (VOE) are a major cause of morbidityand mortality in sickle cell anemia (SCA). Low arginine bioavailability is associated with pain severity and predicts need for pediatric hospitalization (*Morris et al, 2000*). Arginine supplementation has opioid-sparing effects and was found to significantly decrease pain scores in children hospitalized with SCA-VOE compared to placebo in a phase-2 randomized placebo-controlled trial (RCT) performed in the United States (US, *Morris et al, 2013*). Its role to treat acute SCA-related pain in a Sub-Saharan African country is unknown.

Objectives: To determine the role of oral arginine as adjuvant in the management of SCA-VOE.

Methods: A double-blind RCT of oral L-arginine (100 mg/kg/dose every 8 hours until discharge; up to 5 days/15 doses maximum) was performed in children with SCA hospitalized with severe VOE defined as a Numerical Pain Scale Score (PS) of at least 7 on a scale of 0-10, at one of two hospitals in Abuja, Nigeria. All patients received pain management (both opioids and non-opioid analgesics) per institutional practice. Demographics, clinical characteristics, length of hospital stay (LOS), pain scores, time-to-crisis-resolution (*time-to-PS<4*), analgesia medications required, and plasma amino acids levels were obtained before and after supplementation. Mean total analgesic medication quantification scale score (MQS), a sensitive measure to quantify analgesic use in patients with SCA, was utilized as previously described (*Jacob et al, 2007*). Opioid doses were calculated based on morphine equivalents in milligrams (mg).

The study was performed after obtaining the National Agency for Food & Drug Administration & Control (NAFDAC) approval, Ref No. NAF/DER/CT/LAG/2017. The protocol was approved by the Human Research Ethics Committees of the University of the Witwatersrand, University of Abuja Teaching Hospital, Abuja & the National Hospital. The study was registered at the Pan African Clinical Trial Registry (PACTR 201611001864290).

Results: Sixty-eight children with SCA were recruited, aged 5-17 years (mean: 10.6±0.4 years; 35 children randomized into the arginine arm & 33 into the placebo arm). Baseline characteristics were similar between arms (Table 1). MQS was significantly lower in the arginine group vs. placebo (73 [95% CI: 62-84] vs. 120 [97-143]; *p<0.001*). By day 5, 54% of children treated with arginine had been discharged compared to 24% in the placebo arm. Although PS were similar in both groups prior to study drug delivery (8.7±1.1 vs. 8.4±1.2, arginine vs placebo; *p=0.30*), worst reported PS on day 5 were lower in children treated with arginine compared to placebo (1.2±0.4 vs. 3.0±0.5; *p<0.0001*). The mean rate of PS decline was also greater in the arginine arm vs. placebo (1.5 [1.2-1.8) vs. 1.1 [0.9-1.2] cm/day; *p=0.009*). Plasma arginine levels increased by 125% vs 29% in the arginine arm vs. placebo; percent increase in arginine bioavailable inversely correlated with MSQ (r=-0.35;*p=0.02*). There was a non-statistically significant decrease in mean total opioid dose used in the arginine group vs. placebo (3.8 [2.7-4.9] vs 5.1 [3.8-6.5) mg/kg, *p = 0.11)*. Patients receiving arginine had shorter time-to-crisis-resolution (*p=0.0216, Fig1A*), shorter LOS (*p=0.015*; *Fig1B*) and had no serious adverse events. There was 1 death in the placebo group on the second day of admission. There were no differences between groups in development of adverse events, however there was a trend towards more vomiting in the arginine arm compared to placebo (20% vs. 3%, *p=0.07*).

Conclusion: Arginine deficiency plays a role in acute pain requiring hospitalization in Nigerian children with SCA, similar to what has been reported in the US. Plasma arginine levels significantly increased with arginine supplementation, and improved global arginine bioavailability was inversely associated with total analgesia and opioids used in VOE management. Total mean analgesia use and pain scores were lower, while time-to-crisis-resolution and LOS were shorter in children treated with arginine compared to placebo. No serious adverse events occurred in the arginine arm, while rates of adverse events were similar to placebo, providing further support for the safety of arginine therapy in children with SCA. Oral arginine is a promising adjuvant therapy for SCA-VOE management.

Table 1: Baseline Patient Characteristics

Parameters	Arginine group (n = 35)	Placebo group (n = 33)	p	95% CI
Age *(years)*	10.65±3.22	10.50±3.54	0.86	9.82-11.44
Gender				
Male	18 (51.4%)	20 (60.6%)	0.75	-
Female	17 (48.6%)	13 (39.4%)		
Social class				
Upper class	16 (45.7%)	11 (33.3%)	0.54	-
Middle class	10 (28.6%)	8 (24.2%)		
Lower class	9 (25.7%)	14 (42.4%)		
Weight Z	-1.74±1.93	-1.58±1.84	0.73	-0.76-1.08
Height Z	-1.09±1.75	-0.64±1.72	0.29	-0.39-1.29
BMI Z	-1.37±1.77	-2.23±2.89	0.14	-2.0 -0.29
BSA	1.08±0.20	1.10±0.36	0.75	-0.1-0.1
# crisis in preceding year (yr)[F]				
<3	9 (28.1%)	15 (48.4%)	0.25	-
>3	23 (71.9%)	16 (51.6%)		
Hospitalization in previous yr[F]				
<3	25 (78.1%)	27 (87.1%)	0.64	-
>3	7 (21.9%)	4 (12.9%)		
Blood transfusion in previous yr[F]				
0	18 (56.3%)	15 (48.4%)	0.82	
1-2	14 (43.8%)	16 (51.6%)		
Average steady state Hb[F]				
>8g/dl	15 (46.9%)	20 (64.5%)	0.37	-
<8g/dl	17 (53.1%)	11 (35.5%)		
Blood counts at admission				
Red blood cell counts, $x10^{12}$/L	2.70 (2.22-3.15)*	2.70 (2.33-3.30)*		
White blood cell counts, $x10^9$/L	18.2 (14.7-21.4)*	17.8 (14.4-22.9)*		
Platelet counts, x 10^9/L	425 (329-551)*	320 (208-434)*		
Hydroxyurea use				
Yes	5 (14.3%)	5 (15.2%)	1.00	-
No	30 (85.7%)	28 (84.8%)		
Types of crisis				
Vaso-occlusive pain (VOE)	24 (68.6%)	22 (66.7%)	0.96	-
Acute chest syndrome (ACS)	2 (5.7%)	3 (9.1%)		
Mixed crisis (VOE+ACS)	9 (25.7%)	8 (24.2%)		
Baseline pain scores	8.7±1.1	8.4±1.3	0.31	-
Number of pain sites				
1	6 (17.1%)	9 (27.3%)	0.18	-
2-3	24 (68.6%)	14 (42.4%)		
>4	5 (14.3%)	10 (30.3%)		

** reported as median (Interquartile Range); [F] obtained in 32 patients on arginine and 31 patients on placebo*

Figure 1A *(Crisis Resolution = Pain Score < 4)*

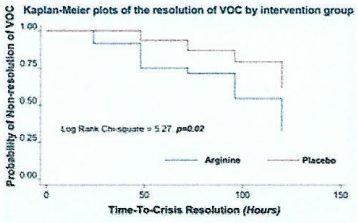
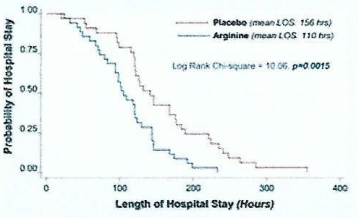


Figure 1B



APPENDIX 5: RCT of Oral Arginine Therapy for Children with Sickle Cell Anemia Hospitalized for Pain in Nigeria

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Randomized Control Trial of Oral Arginine Therapy for Children with Sickle Cell Anemia Hospitalized for Pain in Nigeria

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Short title: Arginine Therapy for Sickle Cell Pain

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Key words: Arginine, sickle cell anemia, Nigerian children, vaso-occlusive pain crisis

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ABSTRACT

Low arginine bioavailability is associated with vaso-occlusive painful crisis (VOC) severity in sickle cell anemia (SCA) and predicts need for pediatric hospitalization. Intravenous arginine therapy has opioid-sparing effects and was found to significantly decrease pain scores in children hospitalized with SCA-VOC in a phase-2 randomized placebo-controlled trial (RCT). Efficacy of oral arginine is unknown. Our objective was to determine the safety and efficacy of oral arginine therapy in Nigerian children with SCA. A double-blind RCT of oral L-arginine-hydrochloride (100mg/kg TID) was conducted in children with SCA-VOC, aged 5-17 years, hospitalized at 2 Nigerian sites. The primary outcome measure was analgesic usage, quantified by difference in the mean Analgesic Medication Quantification Scale (MQS). Secondary outcomes included daily pain scores, time-to-crisis-resolution and length-of-hospital-stay. An intention-to-treat analysis was performed. Sixty-eight children (age 5-17years, mean 10.6±0.4 years; 56% male), were randomized to receive L-arginine (35 patients) or placebo (33 patients). The mean total MQS for the arginine group was 73.4 [95% CI, 62.4-84.3] versus 120.0 [96.7-143.3] for placebo ($p<0.001$). The mean rate of decline in worst pain scores was faster in the arginine arm versus placebo (1.50 [1.23-1.77] vs. 1.09 [0.94-1.24] point/day, $p=0.009$). Children receiving arginine had a shorter time-to-crisis-resolution ($p=0.02$), shorter hospital-stay ($p=0.002$) and experienced no serious adverse event. Pain control was more rapid, total analgesic requirement was significantly reduced, and most notably, time-to-crisis-resolution

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and length-of-hospital-stay were shorter in children with SCA-VOC receiving arginine versus placebo. Given the established safety and low cost, oral arginine is a promising adjuvant therapy for SCA-VOC management.

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INTRODUCTION

Sickle cell anaemia (SCA) is a debilitating hemoglobinopathy affecting 250 million people globally.[1] Africa, and Nigeria in particular, has the largest number of children with this condition. About half of the 300,000 newborns with SCA delivered in the world annually, are born in Nigeria.[2] SCA has remained one of the highest non-infectious causes of mortality in Nigeria; approximately 50-80% of children with this condition die before adulthood and 70% of these deaths are preventable through low cost interventions. [3-5] Pain is the hallmark of SCA and a major contributor to morbidity.[6,7] Vaso-occlusive pain crisis (VOC) is associated with high use of health care resources,[6,8,9] yet there is no effective treatment that targets the pathophysiologic mechanism of acute VOC; symptomatic relief with hydration and analgesics is currently the only available treatment modality.[10-12]

Nitric oxide (NO), enzymatically produced from L-arginine, is a potent vasodilator and exerts multiple effects on vascular and circulating blood cells, including the inhibition of platelet aggregation, down-regulation of adhesion molecules, and modulation of ischemia-reperfusion injury,[13] all pathways adversely affected during VOC.[14] Recently a specific metabolic signature was identified in SCA-VOC that differs from steady-state involving altered arginine-NO metabolism.[15] Low NO bioavailability contributes to vasculopathy in SCA.[16] Arginine, an amino acid, is the obligate substrate for NO production. Low arginine bioavailability in sickle cell disease is associated pulmonary hypertension risk,[17,18] early mortality[17,19] and pain severity,[20-22] and predicts need for pediatric hospitalization.[20] Arginine supplementation has opioid-sparing effects and was found to significantly decrease pain scores in children

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hospitalized with SCA-VOC compared to placebo in a phase-2 randomized placebo-controlled trial (RCT) performed in the United States.[22] Its role to treat acute SCA-related pain in Africa is unknown. The aim of this study is to determine the efficacy and safety of oral arginine therapy to treat children with SCA-VOC in Nigeria.

METHODS

Study Design

We conducted a prospective, double-blinded, two-site, phase-2 RCT trial of oral L-arginine therapy involving children with SCA-VOC with or without acute chest syndrome (ACS), from February 2017-February 2019 in Nigeria. The data were collected and stored in a secure web-based database (REDCap) hosted at the University of the Witwatersrand, Johannesburg. The trial was approved by the appropriate institutional review boards of the University of Abuja Teaching Hospital and National Hospital, Abuja, where the study was performed.

Participants

Children with SCA, aged 5-17 years, admitted into the Emergency Paediatric Unit of the University of Abuja Teaching Hospital and National Hospital, Abuja for severe VOC and/or ACS with Numerical Pain Scale Score of at least 7 on a severity scale of 0-10 were eligible. Children with a known/suspected allergy to arginine, those with kidney disease (serum creatinine >1.2 mg/dL), liver disease (clinical diagnosis of

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hepatitis or serum glutamic-pyruvic transaminase (SGPT) > 3 times the upper limit of normal), a metabolic acidosis (serum bicarbonate < 16 mmol/L) or those with non-VOC pain were excluded from participation. Written informed consent was obtained from all participants' parents or guardians as applicable.

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Sites

The University of Abuja Teaching Hospital and National Hospital are tertiary health centres but also provide primary and secondary care to patients with sickle cell disease. In the Paediatric Haematology Clinic of University of Abuja Teaching Hospital, there are 810 patients on the Clinic Register while the National Hospital Paediatric Haematology Register captures 700 patients. In the last 4-5 years, there was a significant increase in the population of SCA patient largely due to insurgency in the Northern part of the country. Abuja, being the Federal Capital Territory is very safe, thus experienced a mass exodus of people into the region. The large influx of people and the stability of the area made it the best option for clinical trial of this nature.

Randomization and Masking

Randomization number sequences were computer generated by a Good Clinical Practice certified pharmacist, through Microsoft Office Excel. Using a block randomized design (block size of 4), patients were randomized by center in a 1:1 ratio of placebo to L-arginine as soon as they presented to the Emergency Department (ED) or within 6 hours of admission. The pharmacist had no additional

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involvement in the trial. Participants were consented and enrolled by the principle investigator, study co-investigators or study staff.

Procedures

The supplement (L-arginine-hydrochloride) and the placebo (sucrose) were provided in visually identical packets with a pre-determined dose, administered by a blinded pharmacist and/or a nurse. A pharmaceutical grade L-arginine powder (1119-34-2, Cat/Case No. A4599) purchased from Ajinomoto Co. Inc., USA, was administered every 8 hours (100mg/kg/dose) for maximum of 5 days/15 doses or until patient discharge, whichever came first, based on previous studies.[18,22,23] The study drug (arginine or placebo) was dissolved in red grape juice, mixed in an identical fashion and consumed immediately, administered by the hospital staff to insure compliance. The patients were followed up at 24 hours (day 1), and daily (days 2-5) after commencement of the study to capture adverse events, the daily worst and least pain score experienced and the pain intensity at the time of evaluation (current pain). Daily assessment of pain scores as an outcome measure were specifically obtained as part of the study protocol as the use of pain scores is not a routine standard practice at these hospitals. Patients received institutional standard of care therapy. The cumulative dose of analgesics administered was computed daily and scored using the validated analgesic medication quantification scale (MQS)[24] while total opioid intake was computed using morphine-equivalent units/kilogram.[22]

The pediatric units utilized a standard protocol for managing severe pain in patients with sickle cell disease and VOC, which included a parenteral opioid at presentation, alternating with non-steroidal anti-

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inflammatory drugs (ketorolac, diclofenac) for all patients, regardless of study participation. In the University of Abuja (UATH), the commonly used opioid analgesic for severe pains is parenteral pentazocine. This is used in a staggered dose format with intramuscular diclofenac/ketorolac. At National Hospital, parenteral pentazocine is the commonly used opioid alternating with parenteral/suppository diclofenac sodium. Liquid oral morphine is often introduced very early in the treatment to replace pentazocine. However, oral morphine is rarely used in the UATH. Parenteral morphine is not readily available and rarely used if ever at either hospital. When the pain is subsiding, patients are maintained on variable combinations of diclofenac (suppository or tablet), oral ibuprofen tablets, and oral acetaminophen tablets alternating with dihydrocodeine. Intravenous hydration of patients is universal, unless otherwise indicated. Malaria is endemic in North-central Nigeria, hence patients with severe crisis are presumptuously treated with antimalarial medication. Those with fever or features of superimposed infection(s) are giving empirical antibiotics until laboratory results are released and subsequent treated are guided by the laboratory results. Rarely do patients receive adjuvants like diazepam or diphenhydramine.

Outcomes

The primary efficacy end point was the difference in analgesic use, as reflected by mean difference in the MQS score, a sensitive measure to quantify analgesic use in patients with SCA.[24] A summary of how the score is generated based on total analgesics is described by Jacob and colleagues. [24] Specifically the primary outcome measure was calculated as the mean difference in total analgesic use up to day 5 of

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admission, compared between treatment groups by students t-test, as reflected by change in the total MQS score. Secondary outcomes included differences in percentage of analgesic use, change in mean pain scores, time-to-crisis-resolution (defined as time-from-study-drug-randomization to pain score <4), the total length-of-hospital stay across the study arms and re-admission within 7 days of hospital discharge. Crisis resolution was defined as a worst pain score <4 (using the Numerical Pain Rating Scale). Time-to-crisis-resolution in patients with missing end-point data due to early discharge were censored at the actual time of discharge. Death before meeting the criteria for crisis resolution was censored at the latest time-to-crisis-resolution for participants who were discharged alive. Assessment of safety and adverse events was made daily. Adverse events were defined according to the Medical Dictionary for Regulatory Activities version 12.0. The number of individuals experiencing any AE was reported, as was the number of total AE events compared between groups without regard for the number of individuals. Although bloodwork was performed as part of eligibility screening prior to randomization, ongoing laboratory surveillance was not performed due to established safety of oral arginine supplementation.[35] An independent Data Safety and Monitoring Board reviewed the study for data safety. The data was first analyzed with the statistician blinded to the code of the study products before unblinding of the study.

Statistical Analysis

The trial was designed to have 80% power to detect a 50% difference in total analgesic medication use, based on data published by Morris *et al.*[22] With an $\alpha = 0.05$, assuming pooled variance (equality of variance) in calculation of the sample size and a ratio of 1:1, estimated sample size is 68 subjects, with 34

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children with SCA in each group. Although total parenteral opioid usage was used to calculate the sample size, MQS was chosen as a surrogate due to differences in practice between the United States and Nigeria.; patients in Nigerian study centers are commonly given only one or two doses of parenteral opioid at ED presentation for severe VOC, and then rapidly switched over to oral medications, therefore making a reduction in parenteral opioids unlikely with any intervention. MQS score data from Nigeria was not available at the time of the protocol development to assist with generation of a sample size, nor was information about the typical proportion of opioid compared to non-opioid use known. Due to the paucity of data from Nigeria, estimates were made based on published experiences in the US. [22] The primary outcome measure was calculated as the mean difference in total analgesic use up to day 5 of admission, reflected by the total mean MQS score compared between treatment groups by Student's t-test. Data was analyzed, with an "intention-to-treat," using STATA 14 (StatCorp, LP, College Station, TX, USA) and Minitab 17 (Minitab, LLC, State College, Pennsylvania) statistical software. Normality of the continuous variables was assessed using the Shapiro-Wilk test. . The Pearson's correlation coefficient was used to assess the linear relationship between some continuous variables as well as the intensity of pain between the intervention and placebo arms. Scatter plots with line of best fit were drawn to test the relationship between some continuous variables and medication quantification scale scores between the placebo and the intervention arms. The time-to-crisis-resolution, as well as time-to-discharge between the two groups, was compared by using Kaplan-Meier plots and log-rank tests. The total daily and cumulative analgesic intake, as well as the daily decline in pain scores between the two study groups, were analyzed using linear mixed models. The treatment arm was the fixed effect while time was the random effect. A $p<0.05$

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reflected statistical significance. An independent validation of the final database was performed by the Emory University Department of Pediatrics Biostatistical Core.

Trial Registration

The study was approved by the National Agency for Food and Drug Administration and Control (NAFDAC), Ref No:NAF/DER/CT/LAG/2017, by the Human Research Ethics Committees of the University of the Witwatersrand (M 160326), University of Abuja Teaching Hospital, Abuja (Ref No:FCT/UATH/HREC/PR/517) and the National Hospital (Ref No:NHA/EC/090/2016). The study was covered by a AIICO Insurance Policy, (No:310/00001/17/TQ/HO) and it was registered at the Pan African Clinical Trial Registry (PACTR201611001864290).

Role of Funding Source

This study was funded by the Tertiary Education Trust Fund (to RO) and in part by NIH/NCCIH K24AT009893 (to CRM). The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data Sharing Statement

For original data please contact richardonalo@yahoo.com. The study protocol is included as a data supplement available with the online version of this article.

RESULTS

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Children with SCA were recruited into this trial from February 2017-February 2019. The CONSORT flow diagram (Supplement Figure 1) illustrates participant screening and enrollment. A total of 68 children were randomized into this study, 35 receiving arginine and 33 receiving placebo. Patients achieving resolution of crisis were discharged home as soon as possible and discontinued from the study. Forty-one patients received the maximum 15 doses of the treatment. All the patients that discontinued the drugs did so because they were discharged home, except for one patient who died on the second day after commencement of therapy. Baseline characteristics are summarized in Table 1. Clinical markers of disease severity were similar between arms. There was a strong positive correlation between the intensity of pain and number of pain sites by patient report ($r=0.73$, $p<0.001$).

The primary outcome for this trial was the effect of L-arginine supplementation on analgesic use during the course of the hospital stay, as reflected by the difference in the mean total MQS score, compared to those children receiving placebo. Arginine therapy was associated with a lower total MQS score compared to placebo (73 [62.4-84.3) vs. 120 [96.7-143.3]; $p<0.001$), reflecting a 39% reduction in the mean total dose of analgesics required during hospitalization. Analgesic usage was similar in patients on arginine and placebo on day 1, however children receiving arginine had lower total MQS scores compared to their placebo counterparts over time with a mean difference of 46.7 (95% CI [21.8-71.4], $p=0.001$; Table 2). The mean cumulative MQS score began to diverge by 48 hours of treatment initiation, with sustained separation of curves over the duration of the trial (Figure 1A), With further categorization of the analgesics into opioids and non-opioids, patients in the arginine arm had a 26.5% reduction in the total opioid

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requirement when compared to patients on placebo. The mean [95% CI] total dose of opioids (in morphine-equivalents) used was 3.8 [2.7-4.9] vs. 5.1 [3.8-6.5] mg/kg; p=0.11 in the arginine group vs. placebo, respectively. The difference, though not statistically significant, is clinically relevant. Pearson correlation analysis of the study showed a strong correlation between opioid use (total oral + parenteral in morphine equivalents (mg/kg) and total MQS score (r=0.6, p<0.001).

Impact of L-arginine supplementation on intensity of pain: The mean (±SD) pain scores were similar at presentation (8.7±1.1 vs. 8.4±1.3; p=0.30) in the arginine and placebo arms, respectively, but became significantly lower for patients on arginine compared to placebo, demonstrating faster pain reduction after treatment with arginine. The mean worst, and current daily pain scores are shown in Figure 1B and 1C. The mean rate of decline in worst pain scores was more rapid in patients on the arginine arm vs. placebo (1.5 point/day [95% CI, 1.23-1.77] vs. 1.09 point/day [0.94-1.24], p=0.009, respectively.

Effect of L-arginine supplementation on the number of pain sites: The number of pain sites were similar on presentation. The most frequent number of pain sites was 2 in both treatment arms: 37.1% in the arginine arm versus 39.4% in the placebo arm (p=0.60). By day 3 only 8.6% in the arginine arm versus 37.5% in the placebo arm complained of pains in two sites (p=0.02). Of the 10 patients that complained of pain in multiple sites by day 5, only one was in the arginine arm compared to 9 in the placebo arm (p=0.04).

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Time-to-crisis-resolution: The difference in time-to-crisis resolution between the arginine and placebo group was statistically significant with Log-rank Chi-square=5.27, p=0.02. (Figure 2A). A total of 28 (41.2%) patients achieved crisis resolution by the definition of a pain score < 4; 19 (67.9%) of these were in the arginine group while 9 (32.1%) were among those that had placebo. By 72 hours of study, 10 (28.6%) patients in the arginine arm had achieved crisis resolution while in the placebo arm, crisis resolution occurred in 5 (15.2%) patients. By day 5 of the study, 6 (17.1%) patients in the arginine group and 8 (24.2%) patients in the placebo group still had worst pain score of 5 or higher. The mean time to achieving crisis resolution was shorter [75.8±36 hours (95% CI, 63.4-88.2)] in the arginine group than in placebo group [93.3±32.7 hours (95% CI, 81.7-104.9)] by a mean of 17.5 hours (95% CI, 0.84-34.20). Since 40 patients did not achieve "crisis resolution" based on our definition, 59% of patients were censored for this outcome measure.

Length of hospital stay: The median length-of-hospital stay was 105 hours (IQR, 72-144) for patients that received arginine and 141 hours (IQR, 117-205) for patients in the placebo group (Log-rank test p=0.002). (Figure 2B).

Readmission within one week of discharge: Readmissions were similar between study arms. Four (11.4%) patients receiving arginine were readmitted within 7 days of hospital discharge compared to three (9.1%) in the placebo group, p>0.99.

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Concomitant medication: Various medications routinely used in the management of SCA-VOC were used concomitantly with the study drug, including non-steroidal anti-inflammatory drugs, opioid analgesics, intravenous fluids, antimalarial drugs and antibiotics. The drugs used and the frequency of usage was similar in the arginine and placebo arms.

Safety: The rate of adverse events was similar in both treatment groups: 71.4% vs. 78.8%, p=0.79 in the arginine versus placebo arm, respectively. A total of 37 (54.4%) patients reported one or more adverse events, 19 patients in the arginine arm and 18 patients in the placebo arm. There were 31(45.6%) patients who did not report any adverse event (arginine group=16, placebo=15). Most of the patients reporting AEs had only one AE that lasted for a few days and were either treated or resolved spontaneously. A total of 51 AEs occurred, with 25 AEs reported in 19 patients in the arginine arm and 26 AE's reported in 18 patients in the placebo arm. Four cases of serious adverse events (SAEs) were recorded in patients on placebo. Two were cases of empyema thoracis, one case of osteomyelitis and one death which occurred on the second day of admission. The mortality case occurred immediately after an intravenous bolus of diazepam, probably due to respiratory depression. There was however no SAEs in patients that received arginine. (Supplement Table 1). There was a trend towards more vomiting in the arginine arm vs. placebo (20.0% versus 3.0%, p=0.068) that was not statistically significant, but clinically relevant.

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DISCUSSION

This is the first RCT to demonstrate benefits of oral arginine therapy in children with SCA-VOC in sub-Saharan Africa. Arginine supplementation decreased need for analgesic medication, decreased pain intensity faster and enhanced early achievement of analgesia when combined with conventional pain medications compared to placebo. Most notably, total length of hospital stay and time-to-crisis-resolution was significantly reduced.

The total analgesic MQS score, an index that captures all analgesic drugs (opioids and non-opioids) used in the treatment of pain[24] was significantly lower in the arginine group, implying analgesic sparing effect of arginine in pain management. This effect of arginine has previously been documented by Morris *et al*[22] in the setting of children with sickle cell disease hospitalized for VOC where a 54% reduction in parenteral opioid use was observed. Although the reduction in opioid requirement by 27% in the current study was less dramatic than what was reported with intravenous arginine,[22] treatment practices in Nigeria differ from those in the US, with less emphasis on intravenous opioids as primary therapy, making a significant reduction in opioids unlikely. Intravenous arginine may also have a more potent analgesic effect than its oral form. This adds to data supporting the safety and efficacy of supplemental oral arginine in sickle cell disease; a study by Cox et al. reported an excellent safety profile and a potential effect on increased nitric oxide production as assessed by endothelial responses[25,] and through use of non-invasive oral nitrate stable

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isotopic method [26] in a 4-month random-order crossover trial of 2 ready-to-use supplementary food formulations in children with SCD in Tanzania, one of which was fortified with arginine and citrulline.[25,26]

Although NO-related vasodilation from arginine supplementation may contribute to pain relief, arginine is also a precursor of kyotorphin (L-tyrosyl-L-arginine), which is an endogenous analgesic dipeptide.[27,28] Marked analgesia was reported in non-SCD patients with various forms of pain 30-40 minutes after intravenous arginine compared to placebo, with an effect that lasted 6-24 hours. Interestingly, the L-arginine induced pain relief was dose-dependent and blocked by intravenous naloxone [27,28]. A dose-dependent impact on NO metabolite production in children with VOC has been reported with oral arginine,[23,29] and more recently, with intravenous arginine with respect to mitochondrial function and oxidative stress.[30] A decrease in the frequency and intensity of chest pain has been reported by Bortolotti *et al*[31] in non-SCA adult patients with eosophageal motor disorders following oral administration of arginine, thus further corroborating the effects of arginine in management of pain. Similarly, other authors have observed improved analgesia when arginine was co-administered with conventional analgesics[32,33] in addition to sickle-related pain.[22,23,29]

The relevance of the analgesic property of arginine was further demonstrated by the faster time-to-crisis-resolution and by extension, the shorter hospital stays of patients who received arginine compared to placebo in the present study. A mean difference of 46 hours in length of hospital stay between the arginine versus placebo group has both clinical and economic relevance in contemporary patient care.

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The safety profile of this study is comparable to those of Morris *et al* [18,22,34,35] and other authors[14,36] who also documented mainly minor side effects following administration of arginine, and no serious adverse events. Vomiting was common in the arginine group but did not reach statistical significance.

Limitations

This study had some limitations. Firstly, the study was conducted at only two sites within Nigeria, and may not be generalizable. Secondly, the pain scale used is subjective, though validated, and patients' ability to give accurate assessment of pain severity may be skewed by various cultural and psychosocial factors. Not all patients achieved "crisis resolution" based on the set definition of a pain score <4, which is a common challenge with this outcome measure. In addition to SCA, some patients may have been impacted by their nutritional status or the presence of infectious diseases like malaria or HIV, all conditions that impact global arginine bioavailability,[19,37-43] and potential response to arginine therapy. Finally, the specific mechanism(s)-of-action for arginine therapy remains to be determined, although several feasible mechanisms that include NO generation,[23] improvement in mitochondrial function/bioenergetics[30], and decreased oxidative stress[22,44] have been described, in addition to NO-independent mechanisms specifically targeting pain pathways that impact kyotorphin production.[27] It is interesting to note that the recently FDA-approved amino acid L-glutamine is an arginine precursor whose mechanism remains unclear, but likely shares some common pathways with arginine.[14]

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Conclusions

This study demonstrates for the first time that oral arginine therapy improves pain control in children with SCD-VOC and reduces analgesic requirement. Most notably, time-to-crisis-resolution and length of hospital stay were significantly shorter in children receiving arginine. Given the safety, tolerability and low cost of this nutritional supplement, oral arginine is a promising adjuvant therapy for SCA-VOC. A definitive phase 3 trial of arginine therapy is warranted.

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FIGURE LEGEND

Figure 1: Medication quantification scale (MQS) scores and trend in the daily pain scores. **A.** Line plot of cumulative MQS score between the treatment arms showing a lower cumulative MQS score in the arginine group compared to placebo, ($p<0.001$). A mixed effect model was used to test for differences in MQS scores among the treatment arms over time. The trend in the rate of decline in patient reported daily pain scores for the mean reported **B**. *Worst* and **C.** *Current* daily pain scores days 1 through 5 between the treatment arms, with mixed model p-value indicating the difference in the rate of decline. Significant differences are observed for worst and current pain scores in the arginine arm compared to placebo, $p=0.001$, $p=0.008$, respectively, using a Linear mixed model.

Figure 2: Kaplan-Meier plots showing **A**. time-to-crisis-resolution, with significantly shorter hours in the group receiving arginine compared to placebo ($p=0.02$), and **B.** the duration of hospital stay by intervention group showing patients treated with arginine experiencing a shorter length of hospital stay compared to placebo, with a median of 105 hours vs. 141 hours respectively, $p=0.002$. Survival curves were analyzed with a Mantel Hanzel (log-rank) test.

Supplement Figure 1. CONSORT flow diagram trial profile for enrollment and analysis. A total of 458 children with sickle cell anemia and severe vaso-occlusive pain were eligible for participation at 2 sites in Nigeria. Of those, 68 patients were randomized, 35 into the arginine arm and 33 into the placebo arm. All patients received their allocated study drug per protocol.

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Meetings Presented: The American Society of Hematology annual meeting, Orlando, FL, December 2019.

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Data Availability Statement

Data available on request from the authors.

Disclosure Statement

All authors report no conflicts of interests. Claudia R. Morris, MD, is the inventor or co-inventor of several UCSF-Benioff Children's Hospital Oakland patents/patent-pending applications that include nutritional supplements, and biomarkers of cardiovascular disease related to arginine bioavailability, is an inventor/co-inventor several Emory University School of Medicine patent applications for nutritional supplements, is a consultant for Pfizer, and has received research support from the United States Food and Drug Administration, and the National Institutes of Health.

Authorship Contributions

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RO designed research question, wrote the study protocol, obtained funding, obtained informed consent/assent, insured compliance and regulatory oversight, analyzed and interpreted the data and wrote the first draft of the manuscript. **PC** helped designed research question, assisted with revision of the study protocol, and assisted with writing the manuscript. **AC** helped designed research question, assisted with revision of the study protocol, and assisted with writing the manuscript. **BCV** performed and interpreted targeted amino acids analysis, and reviewed the manuscript. **N-AU** assisted with revision of the study protocol and the manuscript. **OOO** helped to secure ethical clearance, assisted with writing the manuscript. **VDO** assisted in measuring the research product powder into dispensable doses, and assisted with writing the manuscript. **YZ** administered the research products to patients, assisted in daily monitoring of the patients, helped in data collection and reviewed the manuscript. **AUA-K** assigned code to the study products, ensured that blinding process is optimal, and reviewed the manuscript. **OMD** performed the statistical analysis and data interpretation, and assisted with writing the manuscript. **JF** performed an independent validation of the final database through the Emory University Department of Pediatrics Biostatistical Core, assisted with data analysis, data interpretation and writing the manuscript. **CRM** helped designed the research question, assisted with revision of the study protocol, helped analyzed and interpreted the data and assisted with writing the manuscript.

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success of this clinical trial. The authors also recognize the significant contributions of the patients with SCA and their families, without whom this study would not have been possible.

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Table 1: Baseline Characteristics in the Intention-to-Treat Population

Parameters	Arginine group (n=35)	Placebo group (n=33)
Age (years)	10.7±3.2	10.5±3.5
Gender – Male	18 (51%)	20 (61%)
Social class		
Upper class	16 (46%)	11 (33%)
Middle class	10 (29%)	8 (24%)
Lower class	9 (26%)	14 (42%)
Weight Z score	-1.74±1.93	-1.58±1.84
Height Z score	-1.09±1.75	-0.64±1.72
Body Mass Index Z score	-1.37±1.77	-2.23±2.89
Body Surface Area	1.08±0.20	1.10±0.30
No of crisis in preceding year$^{\beta}$		
<3	9 (28%)	15 (48%)
≥3	23 (72%)	16 (52%)
Hospitalization in previous year$^{\beta}$		
<3	25 (78%)	27 (87%)
≥3	7 (22%)	4 (13%)
Blood transfusion previous year$^{\beta}$		
0	18 (56%)	15 (48%)
1-2	14 (44%)	16 (52%)
Average steady state Hb$^{\beta}$	7.7±0.9	8.1±1.1
Hb concentration at admission	7.4±1.0	7.1±1.4
White blood cell count, x10^{6}/L	18.2 [14.7-21.4]*	17.8 [14.4-22.9]*
Platelet count, x 10^{6}/L	425 [329-551]*	320 [208-434]*
Oxygen saturation (%)	95.1±3.7	98.3±2.6
Patients with confirmed malaria	6 (17%)	6 (18%)
Hydroxyurea use –Yes	5 (14%)	5 (13%)
Types of crisis		
Vaso-occlusive painful crisis	24 (69%)	22 (67%)
Acute chest syndrome	2 (6%)	3 (9%)
Mixed crisis	9 (26%)	8 (24%)
Baseline pain scores	8.7±1.1	8.4±1.3
Number of pain sites		
1	6 (17%)	9 (27%)
2-3	24 (69%)	14 (42%)
≥4	5 (14%)	10 (30%)

Data are mean (SD), n (%), or median [Interquartile Range]; β obtained in 32 patients on arginine and 31 patients on placebo. Social status was classified by Oyedeji method based on the occupation and level of education.*

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Table 2. Daily Analgesic Medication Quantification Scale (MQS) scores of patients treated with Arginine compared to Placebo

	Arginine		Placebo		
Day of Assessment	**N**	**Mean MQS [95% CI]**	**N**	**Mean MQS [95% CI]**	**p-value**
Day 1	35	16.90 [14.40-19.40]	33	17.77 [14.83-20.71]	0.65
Day 2	33	18.70 (16.36-21.04]	32	21.31[18.39-24.23]	0.16
Day 3	28	17.17 [14.84-19.50]	30	20.46 [17.47-23.45]	0.08
Day 4	24	12.69 [10.54-14.84]	28	18.79 [15.82-21.76]	**<0.001**
Day 5	16	10.72 [8.28-13.16]	25	17.48 [13.59-21.37]	**0.01**
Total		73.35 [62.36-84.33]		120.00 [96.67-143.33]	**<0.001**

Primary analysis: A mixed effect model was used to test for differences in MQS scores among the treatment arms over time and post hoc (least significant difference) tests were run to ascertain which day the difference occurred.

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Figure 1

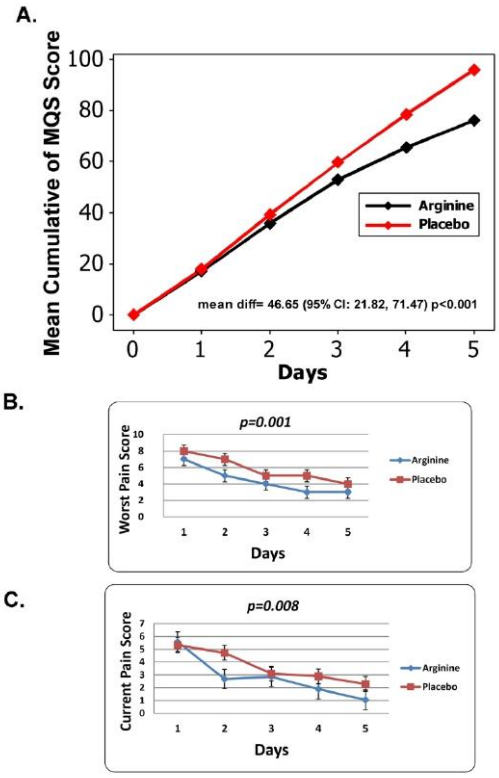


Figure 1 revised MQS and pain scores

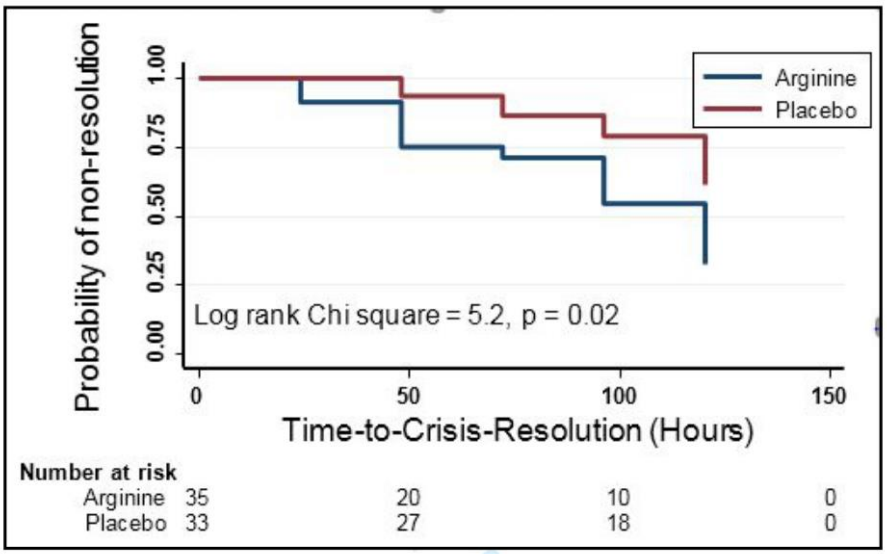
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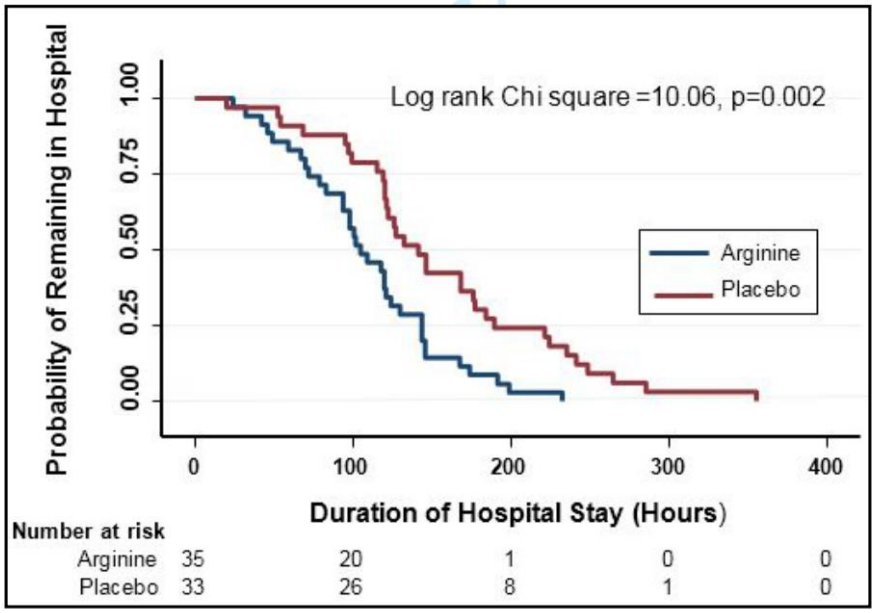
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Figure 2

A.

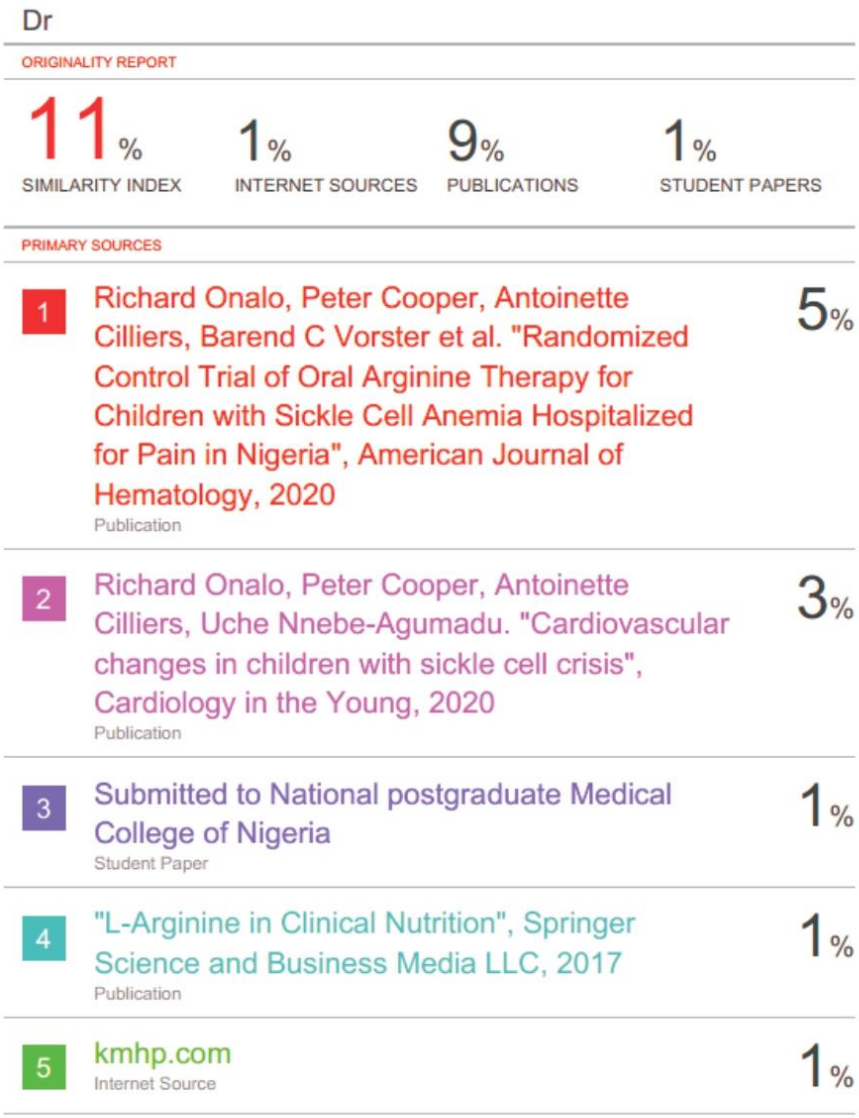


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APPENDIX 6: Turn-it-in Originality Report



Dr

ORIGINALITY REPORT

11%	1%	9%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Richard Onalo, Peter Cooper, Antoinette Cilliers, Barend C Vorster et al. "Randomized Control Trial of Oral Arginine Therapy for Children with Sickle Cell Anemia Hospitalized for Pain in Nigeria", American Journal of Hematology, 2020 Publication	5%
2	Richard Onalo, Peter Cooper, Antoinette Cilliers, Uche Nnebe-Agumadu. "Cardiovascular changes in children with sickle cell crisis", Cardiology in the Young, 2020 Publication	3%
3	Submitted to National postgraduate Medical College of Nigeria Student Paper	1%
4	"L-Arginine in Clinical Nutrition", Springer Science and Business Media LLC, 2017 Publication	1%
5	kmhp.com Internet Source	1%