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Original Article

Impact of oral L-arginine supplementation on blood pressure dynamics in children with severe sickle cell vaso-occlusive crisis

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Abstract: Sickle cell anaemia (SCA) patients generally have lower blood pressures compared to those with the AA haemoglobin genotype. However, during vaso-occlusive crises (SCA-VOC), blood pressures (BP) may elevate transiently to levels beyond the 95th percentile. The risk of stroke or even death increases with increasing systolic BP in SCA. Therefore, interventions targeted at BP reduction may be essential during severe vaso-occlusive episodes. Reduction in BP was achieved with arginine therapy in a meta-analysis of randomized controlled trials (RCT) in non-sickle cell adults. The impact of oral arginine (given for pain control) on the BP of children with SCA-VOC has not been documented. Methods: A double-blind RCT of oral L-arginine hydrochloride as adjuvant therapy for pain reduction was conducted in children with SCA-VOC, aged 5-17 years, over a 2-year period. The mean change in BP and the time to achieve BP <90th percentile was added as part of the outcome variables. The anthropometry, pain scores and mercury sphygmomanometry were done following standard procedures. BP percentiles were generated using the *Fourth Report* guidelines. Differences in the time to normalization of BP in the treatment arms were tested with Kaplan-Meier analysis. Results: Sixty-six children (57.6% male) were randomized into L-arginine (35 patients) or placebo (31 patients) arm. The prevalence of hypertension (BP ≥95th percentile) at presentation tended to increase as the pain scores increased, from a prevalence of 50% in patients with a score of 7 to 65% in those with score of 10 (systolic hypertension) and from 44.4% in patients with pain score of 7 to 50% in patients with pain score of 10 (diastolic hypertension). Patients that received arginine recorded a 12.8±3.2 mmHg decline in mean systolic BP compared to the placebo group, where a mean difference of 7.6±1.5 mmHg was observed, P<0.001. Similarly, the mean diastolic BP reduced by 13% in the arginine group and 7.5% in the placebo group, P<0.001. Children who received arginine tended to achieve BP normalization much faster than the placebo group (P=0.112), and no serious adverse events were documented related to the hypertension or arginine administration. Conclusions: High blood pressure (≥95th percentile) is common amongst children with SCA-VOC and are mostly asymptomatic. Administration of oral arginine given for pain control achieves a reduction of the BP at a faster rate in children compared to placebo and it is safe.

Keywords: Oral arginine, blood pressure, sickle cell anemia, Nigerian children

Introduction

Sickle cell disease (SCD), the commonest haematological disorder globally, is typified by sickle cell anaemia (SCA), the severest form of the disease. Africa, with Nigeria in particular, is the host to more than half of the total world's population of 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) [1]. 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. The changes in RBC rheology increase rouleaux formation and the tendency to intermittent microvascular obstruction and consequently ischaemic injuries to various organs [2-6] which may manifest clinically as stroke, worsening anaemia, pulmonary hypertension, congestive cardiac failure, priapism and excruciating bone pain.

Pain is the hall mark of vaso-occlusive crisis and accounts for about 70% of hospitalizations in SCD patients [7]. The sequence of pathophysiologic events leading to the perception of pain following vaso-occlusion in SCD is still not fully understood. It is however opined 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 [8]. Increased heart rate and increased inotropy are the direct effects of norepinephrine on the heart. Vasoconstriction mediated through postjunctional α_1 and α_2 adrenoceptors occurs in most systemic arteries and veins. The overall cardiovascular response is increased cardiac output and systemic vascular resistance, which results in an elevation in arterial blood pressure.

The blood pressure of patients with sickle cell disease in steady state (absence of acute symptoms), is generally lower than those of haemoglobin S-negative individuals [9-11] due to several reasons including renal tubular dysfunction causing sodium and water wasting [12], autonomic dysfunction resulting in an alteration of the peripheral vascular resistance [13], impaired endothelial response to nitric oxide, reduced vascular reactivity and zinc deficiency [10, 12-14]. Consequently some authors [9] regard a normal blood pressure (BP) in sickle cell patients in steady state to be a hypertensive state. Albeit, it has been documented that 5.4% of children with sickle cell disease in Nigeria [11] and 5.7% of Brazil children [9] with sickle cell disease have systolic or diastolic blood pressures greater than the 95th percentile for age, gender and height. Hypertension in this subset of patients has been associated with strokes and an increase in mortality in the United States [15, 16].

During episodes of sickle cell crisis, the blood pressure can increase acutely to levels above the 99th percentile [11]. This sudden elevation, although transient, may affect autoregulation of cerebral blood flow resulting in an acute cerebral syndrome. Furthermore, acute elevations in blood pressure, by causing an increased afterload, may cause an added stress on an already compromised myocardium that occurs in some patients with sickle cell disease [11]. Therefore, interventions for sickle cell crisis should include measures to limit elevations in blood pressure. These may involve dietary modifications and other non-pharmacological measures.

Dysregulated nitric oxide (NO) metabolism has been linked to elevated blood pressures in patients with sickle cell disease [14]. It is well known that $_L$-arginine is the sole substrate for NO synthesis, therefore administration of arginine to patients with sickle cell crisis may contribute to an increase in NO synthesis and improve endothelium-dependent vasodilation and blood pressure control. A meta-analysis of studies on supplemental arginine in non-sickle cell adults with elevated blood pressure suggests a significant role of arginine in the reduction and even normalization of systolic and diastolic blood pressures [17]. In addition, arginine has been shown to be a useful adjuvant in early achievement of analgesia in children with sickle cell crisis [18, 19]. Adequate analgesia could downregulate the neuroendocrine pathways that mediate BP increase in individuals with pain and thus foster faster normalization of the BP.

The aim of the study was to test the hypothesis that orally administered arginine for the purposes of pain management, lowers blood pressure in children with vaso-occlusive crisis using a placebo-controlled randomized trial format at two sites in north-central Nigeria.

Trial design, participants and methods

The parent trial was designed as a prospective, double-blind, randomized, placebo-controlled, phase II study (RCT) of oral arginine supplementation in children between ages 5 and 17 years with SCA, over a 2-year period. Presence of severe vaso-occlusive crisis (VOC) pain (Numerical Pain Rating Score of ≥7 on a 0-10-point scale) with or without acute chest syn-

drome (ACS) and willingness to participate in the study (with informed written consent and assent) were the inclusion criteria. Patients with osteomyelitis, and pains not characteristics of VOC as well as those receiving bronchodilators, digoxin, antihypertensives, and those that required immediate red cell transfusion or allergic to arginine were excluded from the study.

Approval from institutional research ethics review boards of the University of Abuja Teaching Hospital [FCT/UATH/HREC/PR/517] and National Hospital, Abuja, [NHA/EC/090/2016] where the study was performed as well as the National Agency for Food and Drug Administration and Control [NAF/DER/CT/LAG/2017], were obtained before commencement of the study. All patients provided written informed consent.

The BP, weight and height were measured by the author or a trained nurse according to published standards, after calming the patient soon after admission to hospital. Anthropometric measurements were made with the patient barefooted and wearing as little clothing as possible. A brachial blood pressure was obtained manually in the supine position with an appropriate size cuff using a mercury sphygmomanometer (AccosonDekamet®, A.C. Cossor and Son Surgical Ltd, Accoson Works, Harlow, Essex) applied to the right arm (or left arm for patients with VOC of 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 [20]. The sphygmomanometer's pressure measurement markings corresponding to 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 measurement. The results were interpreted according to normative referenced values [20]. Systolic and diastolic blood pressures were defined as normal when the value was recorded below the 90th percentile for age, sex and height. Blood pressure values between the 90th and less than 95th percentiles were considered to be elevated, while those between 95 percentiles and 99 percentiles plus 5 mmHg were termed stage 1 hypertension and those greater than 99 percentiles plus 5 mmHg were considered stage 2 hypertension [20]. However, in children aged 12 years and above, blood pressures greater than 120/80 mmHg were also considered elevated even if they were recorded to be below the 90th percentile [20].

Blood samples to measure targeted amino acids involved in nitric oxide metabolism namely arginine, ornithine and citrulline were obtained at the same time when intravenous access for medications was achieved. The blood was centrifuged immediately and stored at -80°C until analysis. The targeted amino acids were separated using high performance liquid chromatography and quantified with mass spectrophotometry.

The patients were randomized into an arginine arm and a placebo (sucrose) arm of treatment by a block of 4 randomisation design and the study medications were administered under a double blinded protocol by the study pharmacist at a dose of 300 mg/kg/day in an 8 hourly divided schedule, for five days.

Monitoring

The patients were monitored daily until completion of study protocol or time of discharge, whichever came first. Blood pressures were measured daily in the evenings between 17:00 and 19:00 hours. The presence of adverse drug reactions was also assessed daily. The patients underwent venesection on the 6th day of hospitalization for a repeat analysis of the targeted amino acids. Global arginine bioavailability was computed from the ratio of arginine to the sum of ornithine and citrulline [21].

Statistics

The outcome variables of interest were changes in blood pressure from baseline, the time to normalization of the blood pressure as well as the relationship between elevated blood pressure and indices of arginine bioavailability.

Data was analysed, with an "intention-to-treat", using STATA 14 (StatCorp, LP, College Station, TX, USA) statistical software. Quantitative variables were described as central tendency measures and compared with Student t test. The anthropometric parameters were expressed as

Table 1. Baseline characteristics of patients studied

Parameters	Arginine group (n=35)	Placebo group (n=31)	*P*-value
Gender n (%)			0.562
Male	18 (51.4)	20 (64.5)	
Female	17 (48.6)	11 (35.5)	
Age Mean ± SD (years), range	10.8±3.2, 5-17	10.5±3.6, 5-17	0.858
Weight Mean ± SD (kg), 95% CI	30.3±8.3, 17.1-57.8	31.7±14.5, 15.0-80.0	0.622
Weight z Mean ± SD, 95% CI	-1.8±1.9, -8.2-2.0	-1.6±1.8, -5.3-2.0	0.563
Height Mean ± SD (cm), 95% CI	139.9±15.6, 110-178	141.7±17.5, 108-176	0.659
Height z Mean ± SD, 95% CI	-1.2±1.7, -5.2-3.8	-0.6±1.7, -3.4-4.1	0.203
BMI Mean ± SD (kg/m^2), 95% CI	15.3±2.5, 11.9-23.7	15.2±3.8, 8.5-27.7	0.849
BSA (m^2) Mean ± SD, 95% CI	1.1±0.2, 0.7-1.7	1.1±0.3, 0.7-1.9	0.753
Pulse rate (bpm), 95% CI	101.9, 96.2-107.7	106.3, 100.8-111.8	0.707
Systolic BP (mmHg), 95% CI	122.6, 118.0-127.2	118.9, 114.4-123.4	0.089
Diastolic BP (mmHg), 95% CI	76.4, 71.5-81.4	72.8, 68.3-77.4	0.328
Mean BP (mmHg), 95% CI	92.4, 88.1-97.0	88.9, 84.4-93.1	0.252

BMI = Body Mass Index, BSA = Body Surface Area; SD = Standard Deviation, CI = Confidence Intervals.

Z scores. The proportion of children with high blood pressure at presentation was compared between the two study groups using the Chi square test. The student t test was used to compare the mean of blood pressures between the two study groups. The Pearson's correlation coefficient was used to assess the linear relationship between indices of arginine bioavailability and blood pressure between the intervention arginine 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. Line diagrams were drawn to depict the relationship in the percentage decline in blood pressure from baseline between the two study groups. The time to achieving blood pressure normalization was computed and compared between the two treatment arms using Kaplan-Meier curves. Statistical significance was set at $P<0.05$ for all tests.

Results

Baseline characteristics of the study patients

Of the 66 patients that were randomized, forty-eight (72.7%) received the maximum 15 doses of the treatment. All the patients that discontinued the drugs did so because they were discharged home. The participants consisted of 38 males and 28 females (M:F=1.36:1). The mean age of the patients was 10.6±0.4 years, range 5-17 years. The age, gender and anthropometric characteristics were similar in the arginine and placebo groups (**Table 1**). 45 (68.2%) patients had VOC, while 17 (25.8%) patients had multiple crisis comprising of VOC with severe anaemia (4 cases), VOC with acute chest syndrome (7 cases), VOC with hyperhaemolysis (4 cases) and VOC with acute cerebral syndrome (2 cases). The remaining 4 patients were diagnosed to have acute chest syndrome.

Pattern of blood pressure at presentation

Thirty-two (48.5%) patients had systolic and diastolic hypertension, 4 (6.0%) had only systolic hypertension while another 4 (6.0%) had systolic and diastolic pre-hypertension at presentation. Twenty-six patients (39.4%) had normal blood pressure (systolic and diastolic) at the time of admission. Eleven (16.7%) patients had systolic blood pressure greater than 99th percentile plus 5 mmHg while elevated diastolic blood pressure greater than 99th percentile plus 5 mmHg occurred in 8 (12.1%) patients.

The mean systolic blood pressure was 122.6± 13.3 mmHg (95% CI, 118.0-127.2) in the arginine group and 118.9±12.2 mmHg (95% CI, 114.4-123.4) in the placebo group, P=0.089, while the mean diastolic blood pressure was 76.4±14.4 mmHg (95% CI, 71.5-81.4) in the

Table 2A. Distribution of systolic blood pressure at presentation according to the pain score

Pain scores at presentation	Systolic blood pressure percentiles at presentation				Total (% of N; N=66)
	<90	90-<95	95-99+5 mmHg	>99+5 mmHg	
7	9 (50.0)	0	8 (44.4)	1 (5.6)	18 (27.3)
8	5 (38.5)	0	5 (38.5)	3 (23.1)	13 (19.7)
9	3 (20.0)	2 (13.3)	8 (53.3)	2 (13.3)	15 (22.7)
10	5 (25.0)	2 (10.0)	7 (35.0)	6 (30.0)	20 (30.3)

Table 2B. Distribution of diastolic blood pressure at presentation according to the pain score

Pain scores at presentation	Diastolic blood pressure percentiles at presentation				Total (% of N; N=66)
	<90	90-<95	95-99+5 mmHg	>99+5 mmHg	
7	9 (50.0)	1 (5.6)	8 (44.4)	0 (0.0)	18 (27.3)
8	5 (38.5)	1 (7.7)	5 (38.5)	2 (15.4)	13 (19.7)
9	3 (20.0)	5 (33.3)	6 (40.0)	1 (6.7)	15 (22.7)
10	9 (45.0)	1 (5.0)	6 (30.0)	4 (20.0)	20 (30.3)

arginine group and 72.8±12.5 mmHg (95% CI, 68.3-77.4) in the placebo group, P=0.286.

Pain scores and blood pressure pattern at presentation

Table 2 shows the distribution of blood pressure percentiles according to the pain scores. The proportion of patients with normal systolic BP (SBP <90th percentile) decreased as the pain scores increased, from 50% at a pain score of 7 to 25% at a pain score of 10. In contrast, the proportion of patients with stage 2 hypertension (SBP >99th percentile plus 5 mmHg) increased as the pain score increased, from 5.6% at pain score of 7 to 30% at pain score of 10. However, the latter relationship fell short of statistical significance, z=1.937, P=0.053 (not shown in the table).

Half of the patients with a pain score of 7 had a normal diastolic BP (DBP <90th percentile) and none of them had a DBP >99th percentile plus 5 mmHg. On the contrary, 20.0% of patients with pain score of 10 had stage 2 diastolic hypertension (**Table 2B**). The difference in proportion of children with stage 2 hypertension across the different pain levels was statistically significant, z=2.01, P=0.045. Thus, unlike stage 1 hypertension that showed no predictable pattern with severity of pain, children that presented with the highest pain score (severest pain) were more likely to have marked elevation in systolic and diastolic BP than those with less severe pain.

Correlation between plasma arginine levels with blood pressure of patients with sickle cell crisis

There was a weak negative correlation between plasma arginine levels with systolic blood pressure (SBP), r=-0.242, P=0.070 and diastolic blood pressure (DBP), r=-0.134, P=0.320 at baseline presentation, but the global arginine bioavailability ratio (GABR) failed to demonstrate any pattern of correlation with blood pressure, r=0.053 for SBP and r=0.046 for DBP. The post-supplement arginine levels also maintained the negative correlation with SBP r=-0.316, P=0.037; and DBP, r=-0.280, P=0.048. But unlike the pre-supplement GABR, post-supplementation global arginine bioavailability ratio trended towards negative correlation with SBP, r=-0.225, P=0.194 and DBP, r=-0.175, P=0.314. Overall, plasma arginine levels have an inverse relationship with blood pressure.

Blood pressure changes following $_{L}$-arginine supplementation

The mean systolic and diastolic BP at baseline were comparable between the two groups and are displayed in **Table 3A** and **3B**. The mean systolic BP declined from 122.6±13.3 mmHg on day 0 to 109.8±8.8 mmHg on day 5 of arginine therapy, producing a difference in mean of 12.8±3.2 mmHg (95% CI, 5.7-20.0), which equates to a 11% reduction in systolic BP. In the placebo group, a 6.3% reduction in systolic

Table 3A. Systolic blood pressure dynamics following arginine supplementation

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)	
Day 0	35	122.6±3.3 (118.0-127.2)	31	118.9±12.3 (114.4-123.4)	0.089
Day 1	34	119.4±13.5 (114.8-124.1)	31	117.4±11.7 (113.2-121.6)	0.518
Day 2	33	111.9±11.0 (108.0-115.8)	31	111.1±11.2 (107.0-115.2)	0.779
Day 3	31	108.7±13.3 (104.6-112.8)	31	109.5±21.3 (101.7-117.2)	0.865
Day 4	26	108.8±9.1 (105.1-112.4)	28	108.9±8.4 (105.6-112.1)	0.979
Day 5	22	109.8±8.8 (105.3-114.3)	26	111.4±8.1 (108.1-114.6)	0.515
Difference between day 0 and day 5 BP		12.8 (95% CI, 5.7-20.0)		7.6 (95% CI, 1.9-13.2)	<0.001

Table 3B. 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)	
Day 0	35	76.4±14.4 (71.5-81.4)	31	72.8±12.5 (68.3-77.4)	0.286
Day 1	34	75.8±14.3 (70.8-80.8)	31	73.4±11.6 (69.2-77.6)	0.460
Day 2	33	67.7±10.6 (64.0-71.5)	31	69.6±10.4 (65.7-73.4)	0.484
Day 3	31	65.3±11.4 (61.1-69.5)	31	69.1±11.5 (64.9-73.3)	0.202
Day 4	26	65.2±9.4 (61.6-69.1)	28	68.8±11.2 (64.4-74.1)	0.209
Day 5	22	66.5±7.0 (62.9-70.1)	26	67.4±10.1 (63.4-71.5)	0.626
Difference between day 0 and day 5 BP		11.2 (95% CI, 4.8-17.2)		4.1 (95% CI, -2.2-10.3)	<0.001

BP was achieved, from a mean of 118.9±12.2 mmHg to 111.4±8.1 mmHg [with a mean difference of 7.6±1.5 mmHg (95% CI, 1.9-13.2)] for the same treatment period. The difference in the mean change in systolic blood pressure between the arginine and placebo groups was 5.3 mmHg (95% CI, 4.0-6.5), P<0.001. Similarly, the mean diastolic BP dropped by 13% in the arginine group (from 76.4±14.4 mmHg to 66.5±7.0 mmHg) compared to a 7.5% reduction from baseline (day 0) in the placebo group (from 72.8±12.5, day 0 to 67.4±10.1 mmHg, day 5). The difference in the mean change between the arginine and placebo group (7.2 mmHg) was calculated to be 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.

Changes in proportion of children with high blood pressure (BP ≥95 percentiles) following $_L$-arginine supplementation

The proportion of children on arginine with systolic blood pressure (SBP) ≥95 percentile on day 1 was 60.0%, but this fell to 8.6% on day 5 (**Table 4**). In contrast, 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 was therefore associated with a greater decline in proportion of children with vaso-occlusive crisis-related elevation of systolic blood pressure compared to the placebo, P=0.034 (not shown in table). Analysis of blood pressure measurements on a day-to-day basis showed that 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**).

Elevation of diastolic blood pressures (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. In the placebo group, 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

Table 4. 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 0	<90	10 (28.6)	10 (32.3)	0.487	11 (31.4)	13 (41.8)	0.438
	90-<95	5 (14.3)	1 (3.2)		5 (14.3)	1 (3.2)	
	≥95	20 (57.1)	20 (64.5)		19 (54.3)	17 (54.8)	
Day 1	<90	11 (31.4)	12 (38.7)	0.793	13 (37.1)	16 (51.6)	0.207
	90-<95	3 (8.6)	1 (3.2)		7 (20.0)	2 (6.5)	
	≥95	21 (60.0)	18 (58.1)		15 (42.9)	13 (41.9)	
Day 2	<90	16 (45.7)	11 (35.5)	0.242	18 (51.4)	19 (61.3)	0.010
	90<95	9 (25.7)	5 (16.1)		9 (25.7)	0 (0.0)	
	≥95	10 (28.6)	15 (48.4)		8 (22.9)	11 (35.5)	
Day 3	<90	24 (68.6)	12 (38.7)	0.028	26 (74.3)	17 (54.8)	0.005
	90-<95	4 (11.4)	8 (25.8)		6 (17.1)	1 (3.2)	
	≥95	6 (17.1)	11 (35.5)		3 (8.6)	12 (38.7)	
Day 4	<90	28 (80.0)	17 (54.8)	0.076	28 (80.0)	21 (67.7)	0.095
	90-<95	2 (5.7)	6 (19.4)		5 (14.3)	2 (6.5)	
	≥95	5 (14.3)	8 (25.8)		2 (5.7)	7 (22.6)	
Day 5	<90	29 (82.9)	14 (45.2)	0.006	28 (80.0)	21 (67.7)	0.005
	90-<95	3 (8.6)	7 (22.6)		6 (17.1)	1 (3.2)	
	≥95	3 (8.6)	10 (32.3)		1 (2.9)	9 (29.0)	

with elevated systolic blood pressures required at least 72 hours of arginine administration to have a statistically significant reduction, **Figure 1**.

Decline in pain severity and normalization of elevated blood pressure following arginine therapy

The pain scores declined in both treatment arms with time. 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 however, faster than the rate observed with placebo, 1.09/day (95% CI, 0.94-1.24), P=0.009.

The study has shown that the systolic blood pressure normalizes spontaneously (to values <90th percentile) in both study groups but there was a trend towards achieving normalization in the arginine group earlier than the placebo group. Median time for systolic blood pressure normalization was 96 hours in the arginine group compared to 120 hours in the placebo group, Log-rank Chi square =3.42, P=0.06 (**Figure 2**). The Kaplan-Meier curve for diastolic blood pressure normalization was similar in both groups until 48 hours when the curve began to diverge in favour of arginine and remained divergent until completion of the study. At 72 hours, 87.5% of patients that received arginine had achieved normal BP compared to 62.5% in the placebo group. The observe difference however failed to reach statistical significance, Log-rank Chi square =2.54, P=0.112 (**Figure 2**).

Occurrence of adverse events

Occurrence of adverse events was similar in the arginine and placebo groups. The commonest side effect was vomiting, particularly amongst the arginine group: 20.0% vs. 3.2% in the placebo group, P=0.127. Anaemia warranting blood transfusion occurred in 14.3% of patients in the arginine group and in 15.6% of those in the placebo group, P>0.999. A fever (skin temperature >37.5°C) was recorded in 13.9% and 18.8% of patients on arginine and placebo, respectively. Pleural effusions necessitating chest tube insertion and drainage occurred in two patients that had acute chest syndrome and randomized to the placebo arm of treatment. No serious adverse event was observed in the arginine group.

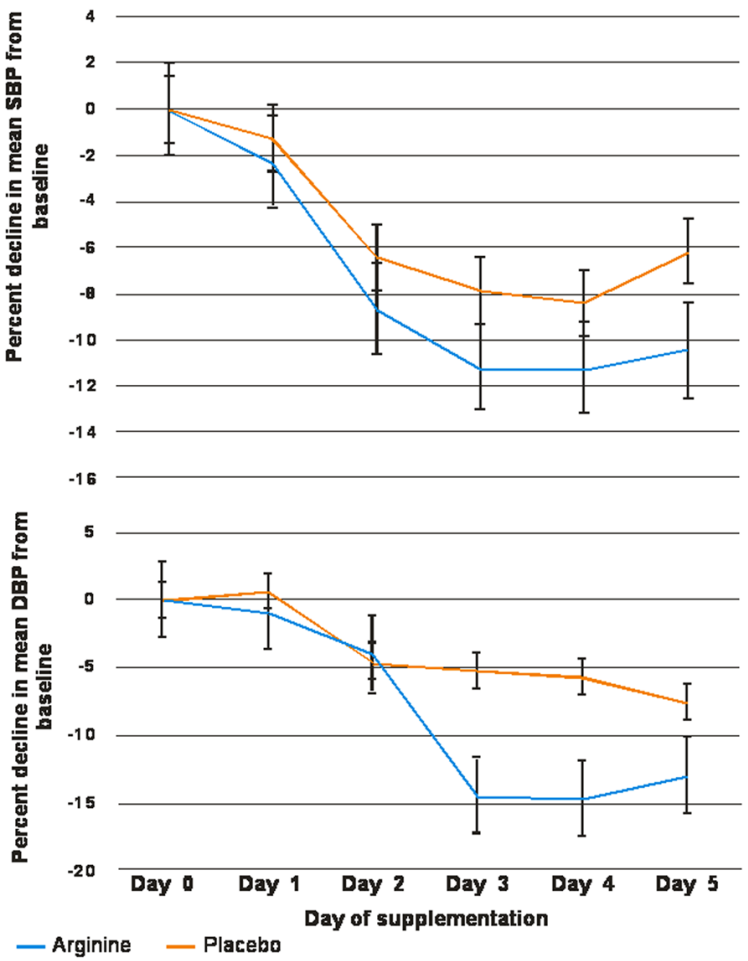

Figure 1. Daily percentage decline in mean of systolic blood pressure (upper panel) and diastolic blood pressure (lower panel) from the baseline in the arginine and placebo groups, showing a steeper rate of decline in the arginine group than in the placebo, *p* value for trend <0.001.

Discussion

Blood pressure (BP) measurements in children with sickle cell vaso-occlusive crisis are usually elevated [11], and may include stage 1 and stage 2 levels of hypertension. In the present study, about half of the patients had elevated blood pressures at presentation, similar to the observation made by Singer *et al* [22] in adult patients with sickle cell crisis. Acute pain causes elevation of blood pressure by two basic mechanisms occurring simultaneously, namely direct stimulation of the sympathetic (autonomic) pathway by electrical pain signals reaching the central nervous system [23] and activation of the neuroendocrine pathway particularly the hypothalamic-pituitary-adrenal axis causing the release of epinephrine and other catecholamine from the adrenal medulla [23-25]. 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 [23, 25]. Another reason for the elevation of blood pressure in sickle cell crisis is the marked increase in total blood volume and cardiac output that characterised the disease [22, 26, 27]. Acute elevations of blood pressures may be asymptomatic but those in excess of the 99th percentile +5 mmHg may alter the regulation of the blood brain barrier resulting in cerebral hyper-perfusion syndromes [11] such as headaches, neurological deficits, and seizures of non-ischaemic origin [28]. Cardiovascular complications such as left ventricular failure, acute aortic aneurysms and stroke [16, 28] may also result from severe acutely elevated blood pressure. In our study, a 10-year old girl had acute cerebral syndrome (stroke) at presentation and a BP of 110/70 mmHg (>90th but <95th percentile for her height), while a boy of 16 years with a BP of 150/100 mmHg (both >99th percentile) had a persistent headache at presentation. Although, the role of BP elevations in their neurologic symptoms was not determined, the documentation of these symptoms in association with elevated blood pressure supports the opinion that acute severe elevation of blood pressure is not innocuous, and should therefore be managed.

Studies [17, 29, 30] in adult patients have reported a reduction in blood pressure levels with the administration of arginine. In the present study, arginine supplementation administered orally in patients with sickle cell vaso-occlusive crisis was associated with lowering of the systolic blood pressure by 5.3 mmHg (95%

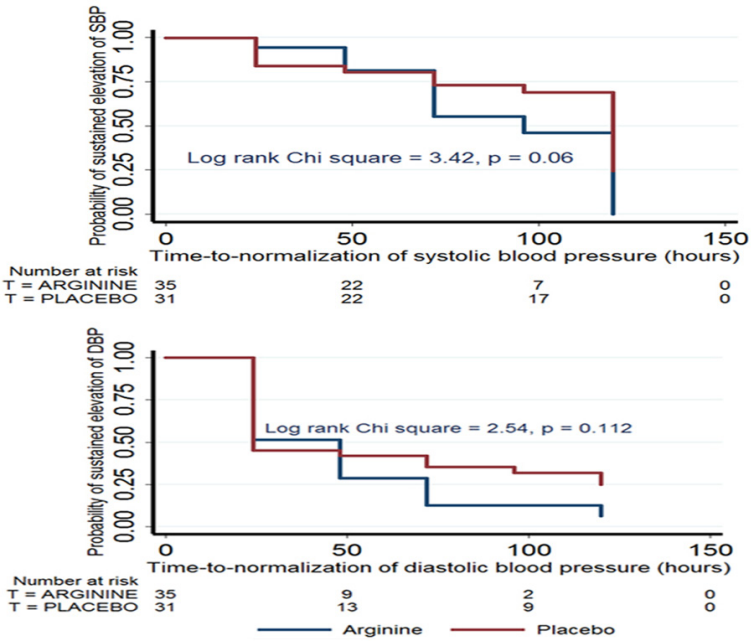

Figure 2. Time-to-normalization of elevated systolic blood pressure (SBP <90th percentile for age, gender and height), and elevated diastolic blood pressure (DBP <90th percentile for age, gender and height). By 72 hours, 50% of patients that received arginine had achieved normalization of systolic blood pressure as against 25% in the placebo group, P=0.06 (upper panel). Similarly, higher proportion of patients (87.5%) in the arginine group than in the placebo group (62.5%) had achieved normalization of diastolic BP, P=0.112 (lower panel) by 72 hours.

CI, 4.0-6.5) and the diastolic pressure by 4.5 mmHg (95% CI, 3.0-6.1). This finding is similar to the report obtained from a meta-analysis of 11 randomized double-blind, placebo-controlled trials involving 387 non-sickle cell adult participants, which showed a significant reduction in systolic blood pressure by 5.4 mmHg (95% CI, 2.3-8.5, *P*=0.001) and diastolic blood pressure by 2.7 mmHg (95% CI, 1.5-3.8, *P*<0.001) [17].

The result of the present study is further corroborated by the report of Pezza *et al* [29] who achieved hypertension control in non-sickle cell adults by addition of oral arginine to conventional antihypertensive therapy after 3 months of failed attempts at normotension with antihypertensive medication. Although the participants of the aforementioned studies are uniquely different by being older and received arginine therapy for several weeks, the association between blood pressure reduction and arginine therapy was clearly demonstrated. Three doses of arginine daily for five days were administered to the patients in the present study together with conventional SCA-VOC therapy and the patients were documented to have a significant reduction in pain intensity and blood pressure measurements. Such short-term arginine therapy has been shown to be effective in achieving endothelial-dependent vasodilation [31] associated with reductions in peripheral vascular resistance and blood pressure in a prospective double-blind randomized crossover study of 10 young men with coronary artery disease who had oral arginine at 7 g three times daily for 3 days. In contrast, other studies have not shown any effect of arginine on the blood pressure of hypertensive and normotensive adults [32].

The mechanism for the arginine-mediated blood pressure reduction in patients is not clear but may include nitric oxide-mediated vasodilation of the renal arteries [33-36] which causes a decrease in renal vascular resistance and inhibition of the angiotensin converting enzyme [34] and consequent normalization of blood pressure. In addition, high plasma arginine concentrations have been reported to improve coronary artery dysfunction in an animal study [37]. There is also a proven linear relationship between plasma arginine levels and NO production following administration of arginine [38]. This linear relationship exists irrespective of the baseline arginine levels and is thought by some to be a paradox since an increase in substrate availability should not alter the rate of NO production due to the low saturation constant for endothelial NO synthase (eNOS) [39]. However, in vitro experimentation confirms the association [40]. Furthermore, overexpression of endothelial arginase 1 enzyme following haemolysis of sickled red cells during sickle cell crisis limits substrate availability for the eNOS because the affinity constant (K_m) of arginine for arginase is much lower than that of eNOS and maximum velocity of reaction (V_{max}) of arginase is 1000-fold higher than that of eNOS

[41]. Therefore, saturating the enzyme system with arginine quickly ensures that the arginase V_{max} is achieved, thus allowing eNOS to function at maximum capacity to replete nitric oxide and cause vasodilation.

Arginine also induces vasodilation through an insulin-signalling pathway whereby the release of insulin from pancreatic beta cells is promoted by arginine [42, 43]. Insulin causes a decrease in plasma dimethylarginine (released in high concentrations from haemolysed sickled erythrocytes during sickle cell crisis) which is an inhibitor of arginine transport and all NOS isoenzymes [44].

In conclusion, blood pressure elevation in patients with sickle cell crisis is common and has a negative correlation with plasma arginine levels. The time to achieving sustained normalization of high blood pressure has been shown to be shorter in patients who are given oral arginine compared to those that do not receive it. This effect may be due to the direct effect of arginine administration on the vasculature, but may also be due to better pain control, and therefore is beneficial in the holistic management of children with sickle cell vaso-occlusive crisis.

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Disclosure of conflict of interest

None.

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