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Original Research Article

Plasma L-Arginine in Sickle Cell Anaemia Patients Attending a Tertiary Health Care Facility in Southwestern Nigeria

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Abstract

Background of Study: Sickle cell anaemia (SCA) is a common monogenetic disorder that is characterized by chronic haemolysis, recurrent vaso-occlusion of the microcirculation and chronic inflammation. Nitric oxide (NO) bioavailability has been found to be impaired in sickle cell disease this is because haemoglobin released during intravascular haemolysis, is a potent scavenger of nitric oxide (NO) and also releases arginase into the plasma. Upon release, arginase metabolizes plasma L-arginine into ornithine, reducing the needed substrate for nitric oxide synthesis and compounding the reduction in nitric oxide bioavailability in SCA. ***Objective***: The aim of this study was to determine the plasma level of L-arginine in adult patients with SCA in steady state and compare with those of age and sex matched normal HbA controls. ***Methods and Materials:*** Thirty adult SCA patients in steady state attending Haematology clinic at University College Hospital, Ibadan, Nigeria and 30 normal HbA age and sex matched controls were enrolled for this study. Haematological parameters were determined by a 5-part autoanalyzer and plasma level of L-arginine was quantified by ELISA method. Data were analyzed and results were considered statistically significant if $p<0.05$. ***Result:*** The respondents consist of 33(55%) females and 27(45%) males with a mean age of 29.4years (±8.9). Haematocrit was significantly higher in controls than steady state, Plasma L-arginine was significantly reduced in SCA patients in steady state than controls. ***Conclusion:*** This study confirmed that there is reduction in L-arginine in SCA patients in steady state compared to normal HbA controls.

Keywords: Sickle Cell Anaemia, haemolysis, vaso-occlusion, L-arginine.

INTRODUCTION

Sickle cell disease is a group of inherited disorders of red blood cells. It is the most common disorder of haemoglobin worldwide. This disease is characterized by the presence of sickle haemoglobin and was the first disease to be characterized at the molecular level but mechanisms underlying the pathophysiology were initially unexplainable [1]. Sickle cell anaemia (SCA) represents homozygous inheritance of haemoglobin S but haemoglobins C, E, D, beta thalassaemia etc form compound heterozygous forms, when they co-exist with haemoglobin S; and are encompassed by the term Sickle Cell Disease (SCD) [2].

Sickle Cell Anaemia results from a point mutation in which adenine is replaced by thymine (GAG to GTG) in the sixth codon of the beta globin gene. This mutation leads to substitution of glutamic acid with valine in the sixth amino acid of the beta (β) globin chain of the haemoglobin molecule. Sickle Cell Anaemia patients have a spectrum of clinical presentations which include recurrent painful vaso-occlusive crises (VOC), stroke, priapism, pulmonary hypertension, acute chest syndrome (ACS) and chronic organ injuries [3, 4].

Arginine is a semi essential amino acid that is derived from dietary protein intake, body protein breakdown, or endogenous de novo arginine production in the kidneys. It becomes an essential amino acid in certain conditions when the capacity of endogenous arginine synthesis is exceeded, these include trauma, sepsis, burns, SCD and thalassemia [5].

Common dietary sources of arginine are meat, poultry, nuts, fish, and watermelon, about 2–7 g of L-arginine is ingested daily in a normal western diet. It is

also a safe nutritional supplement that has been studied in human and animal trials, including a growing number of trials in SCD [5]. Children with SCA have plasma levels that are similar to normal controls. An arginine deficiency develops over time and is influenced by acute events. A state of a low global arginine bioavailability occurs in SCD that goes beyond the concentration of arginine in plasma.

The reduced plasma levels of L-arginine seen in SCD has been found to be associated with increased mortality because low L-arginine level is related to endothelial damage and multiple organ damage [6, 7]. Low level of L-arginine in patients worsens the clinical condition of patients with sickle cell disease. Nitric oxide is a soluble gas with a half-life of seconds, continuously synthesized in endothelial cells from the amino acid L-arginine by isoforms of the nitric oxide synthase enzyme [6].

Recent studies suggest that patients with SCA suffer from decreased nitric oxide reserves [6]. Blood plasma levels of nitric oxide precursors have also been found to be depressed in patients with SCA, particularly during vaso-occlusive crisis and the acute chest syndrome, and these levels vary inversely with pain symptoms [6, 7]. Furthermore, nitric oxide-dependent blood flow is impaired in patients with sickle cell disease [8, 9].

L-arginine has been found to be the precursor for mammalian nitrite-nitrate synthesis [10] and that nitric oxide is the endothelium-derived relaxing factor [11, 12]. In 1988, nitric oxide was identified as the biologically active intermediate product of the L-arginine-nitrite-nitrate pathway in macrophages [13, 14] and endothelial cells [15]. It is now a known fact that many cell types utilize L-arginine to generate nitric oxide, which plays important roles in many diverse processes, including vasodilation, immune responses, neurotransmission and adhesion of platelets and leucocytes [16, 17].

Adult patients with sickle cell disease have been reported to have low plasma L-arginine levels, with subsequent reduction in the bioavailability of nitric oxide, even in steady state [18]. L-arginine is important in the formation of nitric oxide, this has been found to be central in the pathophysiology of the disease and severity of the complications [19], hence the need to estimate the plasma arginine level of these patients in our environment. This study aims to determine the plasma L-arginine levels in Sickle Cell Anaemia in steady state and to compare with those of normal healthy haemoglobin A individuals. The index study hopes to improve our knowledge on the role of L-arginine in the pathophysiology of Sickle Cell Disease among Africans.

MATERIALS AND METHODS

This cross-sectional study consisted of 60 adult individuals enrolled and divided into two groups: Steady state (steady) group made up of 30 SCA patients enrolled during routine follow up visit. Steady state was defined as stable health state in SCA patients who did not have bone pain or any other crisis and no blood transfusions in the previous two months,[20] and Control (HbA) group composed of 30 HbA individuals who were students and workers in the study hospital. The control participants were healthy (HbA) age- and sex-matched adults without previous clinical evidence of haemoglobinopathies. The patients in steady state were diagnosed according to their haemoglobin profile as having homozygous haemoglobin S (HbSS) by alkaline electrophoresis. The control participants were confirmed as having haemoglobin A (HbAA) also by alkaline electrophoresis. The individuals with concurrent overt infection, bone pain crisis, pregnancy, other SCD, and those on hydroxyurea (HU) were excluded.

The attending physicians interviewed all SCA participants, and questionnaires were completed with the guidance of the attending physicians. The questionnaire contained sections on bio-data and past medical history, these were extracted from the patients' case files. University of Ibadan/University College Hospital ethics review committee approved the study (UI/EC/14/0290), and all participants gave written informed consent.

Venous blood was collected from all the participants at the time of presentation to the hospital and dispensed into two EDTA vacutainers. One for analysis of the haematological parameters, the second tube was centrifuged, and plasma was stored in aliquots at -20°C until arginine was assayed. Complete blood count (CBC) was performed using Sysmex XS -1000*i* (Sysmex Corporation, Kobe, Japan), a fully automated 5-part counter. Plasma arginine was quantified using high-sensitivity commercial enzyme-linked immunosorbent assay (ELISA) kits (Span® Biotec Limited, Shenzhen, China) in accordance with the manufacturer's instructions.

Data were analyzed using SPSS version 22.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill.). The descriptive data were presented as means ± standard deviation except otherwise stated. Frequencies were shown in tables and graphs. Results were considered statistically significant if $p<0.05$.

RESULTS

Socio-demographic characteristics of the participants:

*Socio-d*emographic characteristics of the study participants are summarized in Table-1. Of the 60 adults evaluated, there were 30 (11 males and 19 females) SCA patients in steady state and 30 HbA

controls (16 males and 14 females). One-third of the two groups of participants were students.

The haematological parameters of the participants were summarized in Table-2. The control group has the highest PCV, with the mean PCV for the control group being 39.5±4.6.There was a statistically significant difference in the mean values of PCV ($p < 0.001$), WBC ($p < 0.001$), platelet ($p < 0.008$), ANC ($p < 0.001$), ALC ($p < 0.001$) and AMC ($p < 0.001$) between the two groups.

Plasma level of arginine in sickle cell anaemia patients and haemoglobin A controls:

The plasma level of arginine of the two groups were compared in Figure-1. The mean plasma arginine was significantly higher in the HbA control group (88.2±34.6 µmol/L) than in the steady state group (47.3±22.5 µmol/L), $p < 0.001$.

*Pearson Correlation Analysis between Haematological Parameters and L-arginine among the Patients***.** Table-3 shows the correlation between L-arginine and haematological parameters of the sickle cell anaemia respondents. The L-arginine level was significantly associated with the PCV in the sickle cell anaemia group.

Table-1: Socio-demographic characteristics of the respondents

Socio-demographic characteristics			Statistics
	Control (n = 30)	Steady state (n = 30)	
Grouped Age (in years)			
Less than 20	2 (6.7)	3(10.0)	$\chi^2 = 1.79$
20 – 29	14 (46.7)	10(33.3)	df= 4
30 – 39	10 (33.3)	13(43.3)	p = 0.774
40 – 49	2 (6.7)	3(10.0)	
50 and above	2 (6.7)	1(3.3)	
Mean Age			
Sex			
Male	16(53.3)	11(36.7)	$\chi^2 = 1.68$
Female	14(46.7)	19(63.3)	df =14
			p = 0.194
Religion			
Christianity	18(60.0)	22(73.3)	$\chi^2 = 1.20$
Muslim	12(40.0)	8(26.7)	df = 1
			p = 0.273
Occupation			
Student	8(26.7)	10(33.3)	$\chi^2 = 12.36$
Professionals	12(40.0)	8(26.7)	df = 4
Artisans	8(26.7)	4(13.3)	p = 0.015
Petty traders	0(0.0)	8(26.7)	
Unemployed	2(6.7)	0	
Education			
Nil	0(0)	0(0)	$\chi^2 = 0.964$
Primary	4(13.3)	2(6.7)	df = 2
Secondary	8(26.7)	7(23.3)	p = 0.618
Tertiary	18(60.0)	21(70)	

Table-2: Haematological parameters of the respondents

Haematological Parameters	Mean Values ± Standard deviation		P value
	Control (n = 30)	Steady state (n = 30)	
PCV (%)	40.2 ± 4.9	23.2 ± 3.4	< 0.001*
WBC (x 10^3/ µL)	5.266± 1.212	10.704±2.475	< 0.001*
Platelet (x 10^3/ µL)	266.200±50.278	379.133± 120.354	< 0.001*
ANC (x 10^3/ µL)	2.200±1.122	6.746±2.131	< 0.001*
ALC (x 10^3/ µL)	2.232± 613.3	2.659±1.041	0.058
AMC (x 10^3/ µL)	0.458±0.149	1.017±0.512	< 0.001*
MCV (fL)	76.0±19.0	81.1±6.2	0.169
MCH (pg)	27.2±2.2	28.0±2.5	0.188
MCHC (g/dL)	33.7±1.3	34.6±1.8	0.024

* Statistically significant.

PCV- Packed cell volume
WBC- White cell count
PLT- Platelet
MCV-Mean corpuscular volume
MCH- Mean corpuscular haemoglobin concentration
ANC- Absolute neutrophil count
ALC- Absolute lymphocyte count
AMC- Absolute monocyte count
MCH- Mean corpuscular haemoglobin

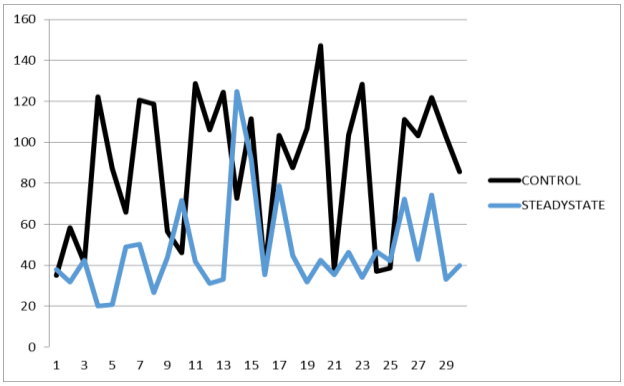

Fig-1: The levels of L-arginine in control and steady state respondents

Independent samples T-test was used

Table-3: Pearson Correlation Analysis between Haematological Parameters and L-arginine among the Patients

	Steady state group
	R (p- value)
ARGININE and	
PCV	4.230 (0.020*)
WBC	0.131 (0.489)
PLT	-0.151 (0.425)
ANC	0.109 (0.567)
ALC	0.160 (0.400)
AMC	0.071 (0.709)

*Statistically significant, Pearson correlation analysis was used.

PCV- Packed cell volume
ANC- Absolute neutrophil count
WBC- White cell count
ALC- Absolute lymphocyte count
PLT- Platelet
AMC- Absolute monocyte count

DISCUSSION

The socio demographic characteristics of the participants in the two groups (controls and steady state) showed no significant differences signifying that the study was not biased. This study also showed that the sickle cell disease in steady state had a significantly elevated leucocytes count than the HbA control group. The major findings of the index study include a significantly reduced mean plasma arginine level in SCA patients compared to arginine level in the controls and no significant association in the haematological parameters except for the haematocrit when L-Arginine was correlated with haematological parameters in sickle cell anaemia group, count noted in the steady state group.

This elevated leucocyte count finding is consistent with the findings of Gonclaves *et al.,* and Kheikhaei *et al.,* which collectively agreed that SCA is a chronic inflammatory disease [21, 22]. it has also been postulated that there is an increased haemopoietic activity and transition of the marginating leucocytes to the circulating pool in SCA, this could be responsible for high leucocyte count noted in steady state group. The finding of a reduced mean arginine level in steady state SCA is similar to other studies that found that SCA patients in steady state were arginine deficient [23, 24]. Arginine is the precursor to nitric oxide, a potent vasodilator which is continuously synthesized in endothelial cells, nitric oxide bioavailability is found to be lower in SCA even in steady state. This logically implies that the reduced arginine level in SCA is as a result of increased consumption of nitric oxide and increased demand for arginine to form the needed nitric oxide. The factors responsible for reduced nitric oxide bioavailability include nitric oxide consumption by free plasma haemoglobin, the free plasma haemoglobin results from chronic haemolysis seen in SCA. It has also been found that free haemoglobin consumes nitric oxide 1000 fold more rapidly in SCA; also it has been found that there is increased reactive oxygen species in sickle cell disease even in steady state. The cause of this increased reactive oxygen species is multi-factorial. Tissue ischaemia results in reperfusion injury and subsequent increase in blood levels of oxygen and oxygen free radicals leading to oxidative damage. SCA patients produce more oxidative species such as hydrogen peroxide and superoxide with impaired free radical defense mechanisms [24]. All these mechanisms subsequently reduce the nitric oxide bioavailability and endothelial dysfunction found in SCA. This however forms the basis for the reduction in the arginine levels in sickle cell anaemia patients even in steady state as the body puts an extra demand on the arginine to compensate for the low nitric oxide in the system.

Limitation of this study, however, is that the influence of genetic modifiers such as haplotypes and fetal haemoglobin levels in the SCA patients were not assessed because of financial constraints. Also, SCA patients in crisis were not studied but the next phase of the study will work on the level of L-Arginine in crisis.

In conclusion, this study has further confirmed sickle cell disease as a clinical entity known to be arginine deficient, most especially in Nigeria with one of the highest prevalence of sickle cell disease. Arginine therapy could be explored to ameliorate this clinical condition.

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