

L- Arginine and Nitric Oxide Levels Among Children with Sick Cell Disease Presented with Vaso-Occlusive Crisis in Federal Teaching Hospital Gombe, Northeastern Nigeria.

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Abstract

Aim: The Aim of This Study Is To Evaluate The Level Of L- Arginine And Nitric Oxide Levels Among Children With Sick Cell Disease presented with vaso-occlusive crisis in pediatric department, Federal Teaching Hospital Gombe, North Eastern Nigeria.

Methology: The area selected for this study was Federal Teaching Hospital (FTH), Gombe, Nigeria. Gombe State is located in the North- eastern part of Nigeria. This study included 60 children aged 1-14 years with a confirmed HbSS electrophoretic pattern presented to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. About 5 millilitres of blood sample was collected from each subject aseptically using the venepuncture technique. The blood was collected into tubes containing dipotassium ethylenediamine tetra-acetic acid (K2EDTA) anticoagulant and the remaining samples was left undisturbed at room temperature and later centrifuged at 3000rpm for 5minutes to obtain clear non- haemolysed plasma. The plasma was transferred into sterile labelled test tubes and assayed (in batches) for L-arginine and Nitric Oxide.**Results:** This study included children with a confirmed HbSS electrophoretic pattern aged 1-14 years with mean age 7.4500 ± 0.50613 years, Presented to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. There was a statistically significant difference between the baseline and post L-arginine supplementation. The L-arginine and nitric oxide were significantly higher post supplementation compared to baseline levels among sickle cell disease subjects with vaso-occlusive crisis ($p= 0.001$ and 0.01 respectively). **Conclusions:** L-arginine and nitric oxide are significantly low in children with sickle cell disease in vaso-occlusive crisis. The study also shows that there is no significant different in relationship between gender and levels of NO and L arginine in children with SCA

Keywords: L-arginine, nitric oxide, sickle cell disease, Vaso-occlusion crisis

INTRODUCTIONS

Sickle cell disease is a global public health problem. As of 2013 about 3.2 million people have sickle-cell disease with 176,000 deaths. Sickle cell disease (SCD) is an inherited haemolytic anaemia whose clinical manifestations arise from the tendency of the haemoglobin (HbSS) or sickle haemoglobin to polymerize and deform red blood cell into the characteristic sickle shape. It is an autosomal recessive disorder and the most common genetic disease affecting African-Americans (Stuart and Nagel 2004). An arginine deficiency develops over time in patients with SCD and is influenced by acute events (Morris *et al.*, 2000). Normal arginine metabolism is impaired through various mechanisms that contribute to endothelial dysfunction, vaso-occlusion and early mortality (Morris, 2014). A single dose of arginine given to patients with sickle cell disease and acute pain episodes resulted in a significant dose-dependent increase in plasma nitric oxide concentration (Morris *et al.*, 2013). Vaso-occlusion is believed to be the root cause of sickle cell pain. Nitric oxide (NO) is a potent vasodilator that plays a role in the vaso-occlusive complications of SCD (Moncada and Higgs 1993). NO is produced in the endothelium from its obligate substrate L-arginine, which is converted to citrulline by a family of enzymes; the NO synthases (NOS) (Kaul *et al.*, 2000). Plasma arginine concentration decreases significantly in both adults and children with vaso-occlusive episode (VOC) and is associated with low NO metabolite (NO_x) levels The lowest arginine levels were found in children requiring admission for VOC, with arginine levels returning to baseline during convalescence in the hospital. Of interest, low plasma arginine alone was a sensitive predictor for admission, while NO_x levels were not, and suggesting a function for arginine bioavailability in VOC severity that goes beyond NO (Morris *et al.*, 2000). A deficiency of nitric oxide, a potent vasodilator, has been identified in sickle cell disease and may contribute to episodes of vaso-occlusive crisis. NO induces a coordinated program of cellular events that promote blood flow, primarily by

suppressing platelet aggregation, expression of cell adhesion molecules on endothelial cells, and secretion of procoagulant proteins (Reiter *et al.*, 2002). Painful episodes of vaso-occlusion are the leading cause of hospitalizations and emergency department visits in sickle cell disease, and are associated with increased mortality. Low nitric oxide bioavailability contributes to vasculopathy in sickle cell disease (Morris *et al.*, 2000).

Since arginine is a building block of nitric oxide, researchers hypothesized that arginine could be beneficial in the treatment of pains related to sickle cell disease. L- arginine is deficient in sickle cell disease.

STATEMENT OF THE RESEARCH PROBLEM AND JUSTIFICATION OF THE STUDY

Sickle cell disease (SCD) is the most common inherited disease among African Americans and affects approximately one out of every 500 newborns (Orki and Higgs, 2010). Nigeria has the largest population of people with sickle cell disease in the world with around 150,000 babies born with this serious condition every year. Episodes of pain, chronic haemolytic anaemia, and severe infections are some of the common characteristics of this disease that begin in early childhood (Orki and Higgs, 2010). Patients with sickle cell disease are deficient in nitric oxide. This lack of nitric oxide constricts blood vessels and causes pain in sickle cell diseases. Most children with sickle cell disease in Africa suffer relentless pain, experience numerous infections and die before their fifth birthday. The optimizing the levels of l-arginine and NO may well help as a very good way of reducing the severity of crisis.

MATERIALS AND METHODS

Study Area

The area selected for this study was Federal Teaching Hospital (FTH), Gombe, Nigeria. Gombe State is located in the North- eastern part of Nigeria; it occupies 18,768 square kilometres and is situated along latitude 10.2500N, and longitude 11.1667E. As of 2006, it had an estimated population of more than 2,353,000 (NPC, 2007). It shares its borders with Yobe state to the North, Borno State to the East, Adamawa State to the South and Bauchi state to the West, with an annual average temperature of 27.0 °C (81°F). Gombe is warm to hot and humid.

Study Population

This study included 60 children aged 1-14 years with a confirmed HbSS electrophoretic pattern presented to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State.

Study Design

This research is a classical experimental case-control study on children with SCA. children with SCA. Age and gender- matched children who were homozygous for haemoglobin S was monitored as controls. This study included children with SCA aged 1-14 years which were randomly grouped into the treatment and age and gender- matched controls individual with SCA in the non-treatment arm was included as controls. Sixty sickle cell anaemic subjects were recruited for the study which comprise of 20 sickle cell subject, 20 sickle cell subjects as control and 20 sickle cell subjects with vaso-occlusive crisis. Quantitative data was gotten by estimating the baseline haematological parameters, serum l-arginine, Nitric Oxide, Total antioxidant capacity (TAC), malondaldehyde (MDA), Glutathione (GLUT) and Pantothenic acid.

Sample Size Determination

Sample size was calculated using the formula: $n = Z^2pq/d^2$

Where n = minimum required sample size in a population $>10,000$

Z = standard normal deviation

P = proportion of success or prevalence

q = proportion of failure ($1-p$)

d = precision, tolerable margin of error, expected difference

Thus $n = Z^2pq/d^2$

$Z = 95\%$ (1.96)

$P = 2.3\%$ (0.0239) (Adewojin *et al.*, 2015).

$Q = 1 - 0.0239 = 0.9761$

$D = 5\%$ (0.05) Therefore $n = (1.96)^2 \times 0.0239 \times 0.9761 / (0.05)^2$

Minimum Sample size = 35

Sampling Techniques/Selection of Subjects

Inclusion Criteria:

This study included confirmed homozygosity for haemoglobin-S, aged (1-14years) and willingness of parents/guardians who offered a written informed consent for their children to participate in this study.

Exclusion Criteria:

The following was excluded from the study; subjects not homozygous for HbS, children < 1 year old and >14 years and children whose parents or guardian refused to provide written informed consent for their children to participate in the study.

STUDY INSTRUMENT

Questionnaire:

Questionnaires were distributed to all consenting participants for the collection of socio-demographic and other biodata such as age, gender, area of residence, any medication, signs and symptoms of diseases.

Ethical Consideration:

Ethical approval was obtained from the ethical committee of Federal Teaching Hospital Gombe (FTHG).

Supplementary Method:

Enrolment = 60 children with SCD

Age eligible for the study = SCD Children (1-14yrs).

Gender eligible for the study = both male and female

Drugs = L- Arginine supplement 350mg

Dose = the dose administered depended on the weight of the children. Subject were given 2-10 capsules two times daily (patient with low weight will be taken lower dose).

This study enrolled 55 children with SCD. Participants were randomly assigned to receive twice daily doses of either a low dose of arginine or a high dose of arginine and for 8 weeks. Five millilitres (5mls) of blood was collected from the patients at baseline before commencement of the treatment. The baseline samples were examined for the following; Nitric oxide level, L-

arginine level, Also, repeating the laboratory parameters nitric oxide level and l-arginine level that were evaluated at baseline as well as the evaluation of the clinical outcome of the supplementation.

Sampling Techniques:

About 5 millilitres of blood sample was collected from each subject aseptically using the venepuncture technique. The blood was collected into tubes containing dipotassium ethylenediamine tetra-acetic acid (K2EDTA) anticoagulant. Samples was left undisturbed at room temperature and later centrifuged at 3000rpm for 5minutes to obtain clear non-haemolysed plasma. The plasma was transferred into sterile labelled test tubes and assayed (in batches) for l-arginine and Nitric Oxide.

Statistical Analysis:

Statistical analysis was performed using statistical package for social sciences (SPSS) version 20.0. Frequencies and percentages were calculated. Student t- test (independent t test and paired sample t-test) and ANOVA was used for comparison of data. The result was presented as mean $\pm$ standard error of mean. A p- value of ≤ 0.05 was considered as significant in all statistical comparisons

RESULTS

This study included children with a confirmed HbSS electrophoretic pattern aged 1-14 years with mean age 7.4500 ± 0.50613 years, Presented to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State

Table 1.1 showed the Summary of the Physical Characteristics of the subjects with mean age 7.4500 ± 0.50613 years, with Mean 131.02 ± 2.72854 height and mean 25.2500 ± 1.07577 weights

Table 1.1: Summary of the Physical Characteristics of the Subjects.

Parameter	Mean $\pm$ SE
Age (yrs) (1-14)	7.4500 ± 0.50613
Height (cm)	131.02 ± 2.72854
Weight(kg)	25.2500 ± 1.07577

Key

Data are presented as Means $\pm$ SEM,

Cm=centimetre,

Kg=kilogram.

Yrs=years

Table 1.2 Effect of L-Arginine Supplementation on SCD Children in VOC stage.

Parameter	Before supplementation (Mean± SE)	After supplementation (Mean ±SE)	p-Value	Remark
L-arg(μmol/L)	8.0977±0.87841	16.9254±2.46022	0.001	S
NOx (mml/l)	26.7 ± 1.7	36.7 ± 2.0	0.01	S

Key

L-ARG = L-arginine

NO = Nitric Oxide

Table 1.3: Relationship between Gender and levels of Nitric Oxide and L- arginine levels among Subjects.

Parameters	Female	Male	P-Value	Remark
L-ARG	7.7150± 0.90965	11.090±02.9380	0.272	N/S
NO	70.3048±12.93713	74.1448±8.37284	0.807	N/S

CONCLUSIONS

Normal arginine metabolism is impaired through various mechanisms that contribute to endothelial dysfunction, vaso-occlusion and early mortality. L-arginine and nitric oxide are significantly low in children with sickle cell disease in vaso-occlusive crisis. The study also shows that there is no significant different in relationship between gender and levels of NO and l arginine in children with SCA.

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