Original Research Article

The Role of Nitric Oxide in Vaso-occlusive Crisis in Sickle Cell Disease Patients in Ghana

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Background: **Recent clinical and experimental data suggest that nitric oxide (NO) may play a role in the pathogenesis and therapy of sickle cell disease (SCD) by maintaining the normal vasomotor tone. However, limited data exist in Ghana and it is therefore imperative that data is collated in that regard.** *Aim and objectives:* **To determine the levels of NO in HbSS and HbSC patients in steady state, in VOC, and in the immediate post-crisis period.** *Materials and method:* **A cross-sectional study was done on 111 SCD patients at steady state, 112 SCD patients in VOC and 67 SCD patients at the immediate post crisis period, all aged 15 to 65 years, with a laboratory diagnosis of SCD at the Sickle Cell Clinic of the Korle-Bu Teaching Hospital with age -matched 102 healthy controls (HbAA) blood donors recruited from the Center for Clinical Genetics and Accra Area Blood Centre at the Korle-Bu Teaching Hospital, Accra.** *Results:* **The mean NO value of (8.66 ± 0.75μm) in control subjects were not significantly different from the mean level of (7.62 ± 0.06μm) in steady state (P>0.05). During VOC however, there was a significant reduction in mean NO levels to (2.08 ±0.81μm) (P<0.05). Mean NO levels rose significantly to (11.97 ± 1.68μm) (P<0.05) in the immediate post crisis period.** *Conclusion:* **Nitric oxide levels were diminished during VOC and rebound significantly during the immediate post crisis period in SCD patients**

Keywords: Sickle cell disease, Nitric oxide, Vaso-occlusive crisis

INTRODUCTION

Nitric oxide (NO) is one of the potent vasodilators known (Moncada & Higgs 1993, Kam & Govender, 1994, Kiechle & Malinski, 1993, Palmer *et al.,* 1987, Claudia *et al.,* 2000, Gladwin *et al., 2004*). Its substrate, L-arginine, is catalyzed by a family of enzymes, the nitric oxide synthetases (NOS) via the L-arginine – nitric oxide pathway (Moncada & Higgs 1993, Kam & Govender 1994, Kiechle & Malinski 1993, Guyton, 1996, Ignarro 1998). A deficiency of L-arginine, the inefficiency of its enzyme and NO unavailability remain the leading factors of vaso-occlusion (Claudia *et al.,* 2000).

The relative levels of this important vasoactive factor, NO, during various clinical phases of sickle cell disease (SCD) patients remain unclear (Gladwin, 2003). **Other studies have** suggested that NO metabolites (NOx) levels are high in patients with SCD (Rees & Grimwade, 1995). Morris and others also found NOx and L-arginine levels being reduced, particularly during VOC and the acute chest syndrome (Morris *et al.,* 2000).

Vaso-occlusion (VOC) is central to the painful crises and acute and chronic organ damage in SCD. Abnormal nitric oxide-dependent regulation of vascular tone, adhesion, platelet activation, and inflammation contributes to the pathophysiology

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of vaso-occlusion (Weiner *et al.,* 2003). Sickling and/or hypoxia associated with SCD may shift the balance in favor of vasoconstriction.

Recent studies also identified elevated cell-free Hb resulting from increased hemolysis in SCD as an important modulator of NO and vascular function (Reiter *et al.,* 2002, Gladwin *et al.,* 2003). Reiter and colleagues demonstrated increased cell-free Hb in steady-state SCD patients and speculated that acute scavenging of NO by oxyhemoglobin may contribute to vasoconstriction and endothelial dysfunction (Reiter *et al.,* 2002). However, the relative levels of this important vasoactive factor NO, during various clinical phases of SCD remained unclear. Hence, data is needed in that regard – thus, the focus of the current research.

PATIENTS AND METHODS

Study design

This was a cross-sectional case-control study.

Target Population and Study site

Samples were drawn from a population of adult SCD patients between the ages of 15 to 65 years who attended clinic at the Center for Clinical Genetics (Sickle Cell Clinic) Korle-Bu Teaching Hospital, Accra, Ghana between the months of January and September 2005.

Inclusion criteria

Sickle cell Disease patients aged between 15 and 65 years who have not used nitrite or Nitrate-containing medications at least two weeks before the sample was taken, were included.

Exclusion criteria

Patients with renal failure (serum creatinine >2 mg/dL), pulmonary edema, cardiogenic shock, history of myocardial infarction in the last 6 months, diabetes mellitus, congestive heart failure, a cerebrovascular accident in the last 6 months, acute asthma, or angina pectoris were excluded.

Patients selection Criteria

An established laboratory diagnosis of SCD was necessary for eligibility for enrollment. Steady state was clinically defined as a patient who has been well and has not been in crisis for at least 2 weeks. Vaso-occlusive crisis was clinically defined as pains in the bones, muscles and joints not attributable to any other cause and requiring parenteral analgesia and admitted in the Centre for some hours. Post-Crisis was clinically defined as a period when patient no longer experiences the VOC pains 3 days after the parenteral analgesia and is certified by a doctor as healthy.

Selection of controls (HbAA)

Voluntary blood donors at the National blood bank, Korle-bu Teaching Hospital were recruited. Blood samples were screened for sickle cell hemoglobin and those with sickle cell trait (HbAS) as well as HbAC were excluded.

Laboratory Assessment

One means to investigate nitric oxide formation is to measure nitrite, which is one of two primary, stable and nonvolatile breakdown products of NO. This assay relies on a diazotization reaction that was originally described by Griess in 1879 (Griess, 1879). The Griess Reagent System is based on the chemical reaction which uses sulfanilamide and *N*-1-**naphthyl** ethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions. This system detects Nitrite in a variety of biological and experimental liquid matrices such as plasma.

Determination of Nitrite Concentrations in Experimental Samples

The average absorbance value of the triplicates of each experimental sample was determined. The nitrite concentration (Y) in µM of each experimental sample was determined by comparison to the Nitrite Standard reference curve. In this case the formula: $Y = 0.0185X + 0.106$, as generated from the standard curve was used, where X is the average absorbance of the experimental sample.

Data Analysis and Presentation

Results were presented as mean ± S.E.M for NO levels. Statistical analysis was done using one – way analysis of variance for four separate observations followed by a post hoc test to test for significant **difference** between groups. Statistical significance was considered at $P<0.05$. The above statistical analyses were achieved using the Statistical Package for the Social Sciences (SPSS) version 10.00 and the MegaStat (TM), 7.25 versions.
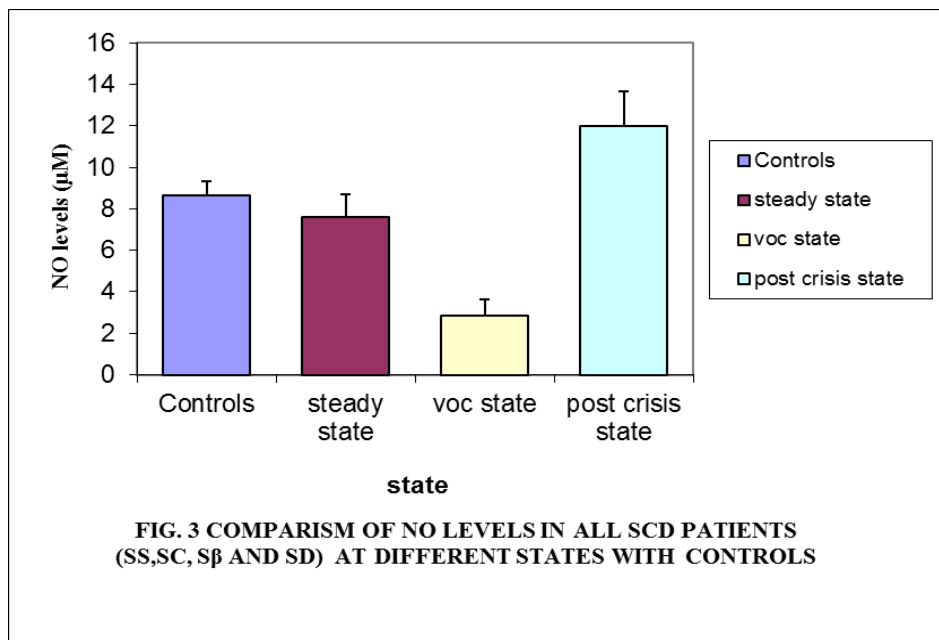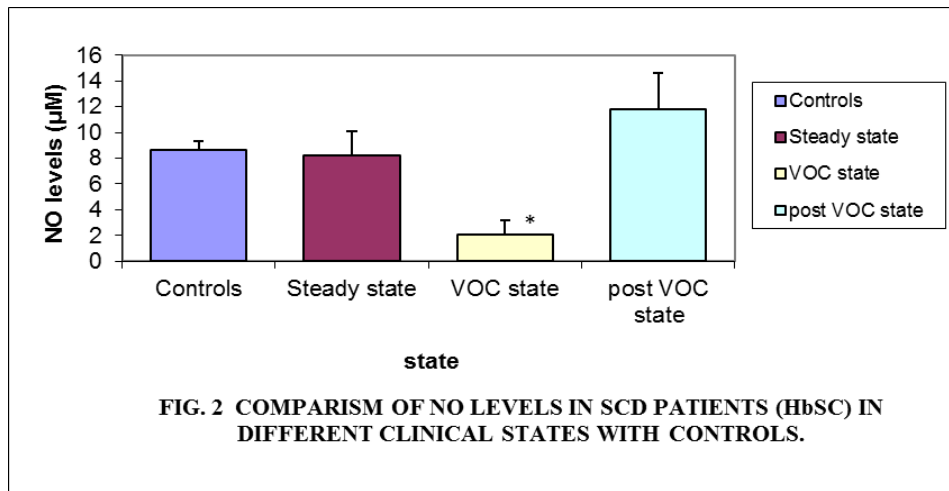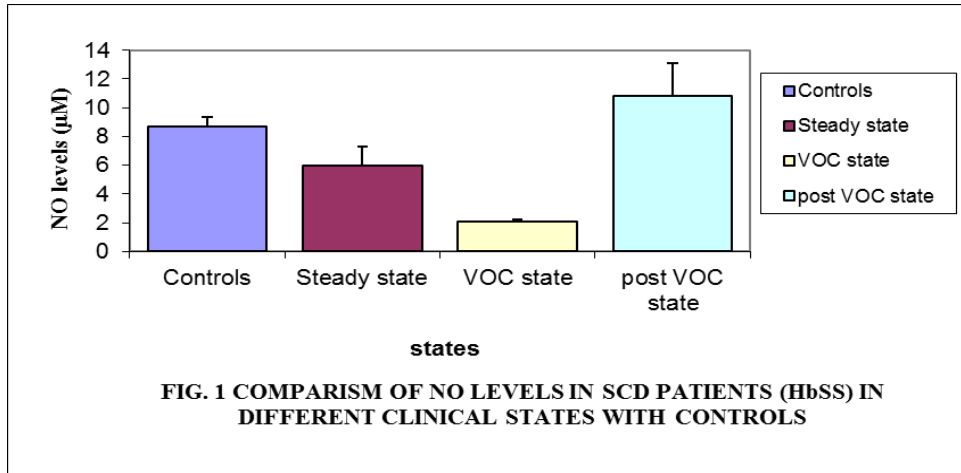
RESULTS

Trend of NO level in different states of SCD patients (HbSS)

We found in multiple comparison that 54 SCD patients (HbSS) in steady state had a mean NO level of (6.00 ± 1.29) µM, 65 SCD patients (HbSS) in VOC had a low mean NO level of (3.07 ± 1.15) µM, 40 of these patients who were followed up to post crisis period had a mean NO level of (10.85 ± 2.28) µM as against 102 controls with mean NO level of (8.66 ± 0.75) µM. (Figure 1) the difference was highly significant with ($P=0.021$). The post hoc analysis showed that there was no significant difference in NO levels between the controls and SCD patients in the steady state ($P = 0.12$) and post crisis state ($P =0.18$). But there was a significant difference between the NO levels during VOC and the steady and post crisis state as well as the controls ($p < 0.001$).

NO trend in different states of SCD patients (HbSC)

Forty-six (46) SCD patients (HbSC) in steady state had a mean NO level of (8.20 ± 1.87) µM, 39 SCD patients in VOC had a mean NO level as low as (2.09 ± 1.09) µM, 24 of these patients who were followed up to post crisis period had a mean NO level of (11.77 ± 2.09) µM (Figure 2) as against 102 controls who had a mean NO level of (8.66 ± 0.75) µM. The difference was highly significant with $P = 0.0005$.

The post-hoc analysis showed that there is no significant difference in NO levels between the controls and the steady state ($P = 0.954$) and post crisis state ($P =0.065$).

FIG. 1 COMPARISM OF NO LEVELS IN SCD PATIENTS (HbSS) IN DIFFERENT CLINICAL STATES WITH CONTROLS

FIG. 2 COMPARISM OF NO LEVELS IN SCD PATIENTS (HbSC) IN DIFFERENT CLINICAL STATES WITH CONTROLS.

FIG. 3 COMPARISM OF NO LEVELS IN ALL SCD PATIENTS (SS,SC, Sβ AND SD) AT DIFFERENT STATES WITH CONTROLS

But there was a significant difference between the NO levels in VOC and the steady and post crisis state as well as the controls (p < 0.001).

Comparison of NO trend in SCD genotypes (HbSS, HbSC, HbSβ and HbSD) in different states with controls.

111 SCD patients in steady state had a mean NO level of (7.62 ± 1.06) µM. 112 SCD patients in VOC had mean NO level of (2.83 ± 0.81) µM and 67 of these patients who were followed up to post crisis period had a mean NO level of (11.97 ± 1.68) µM (Figure 3) as against 102 controls had a mean NO level of (8.66 ± 0.75) µM. The difference was highly significant with p < 0.001. The post hoc analysis showed that there is no significant difference in NO level between the controls and the steady state (P = 0.438) and post crisis state (p =0.079). But there was a significant difference between the NO level in VOC and the steady and post crisis state as well as the controls (p < 0.001).

DISCUSSION

The results of the present study **were** consistent with earlier reports (Enwonwu *et al.*, 1990; Lopez *et al.*, 1996, 2000; Stuart *et al.*, 1999 Claudia *et al.*, 2000; Morris *et al.*, 2000) that the severity of VOC may be related to the degree of depletion of NO. These authors had shown that elevated NO levels were associated with low pain scores whilst lower NO levels were associated with high pain scores.

It was clear that NO participated in the compensatory response to chronic vascular injury in patients with SCD. During VOC, it was expected that NO will play its compensatory role in maintaining vascular function by rising to reverse the occlusion. So although lack of NO may not be the actual cause of VOC, its presence will ameliorate the VOC if not prevent it entirely. Therefore, failure of the NO compensatory system to operate during VOC due to lack of NO bioavailability aggravates the crisis.

During the process of VOC the balance of local vasoconstrictors and vasodilators like NO is altered in favor of vasoconstriction. This may account for the vasoconstriction that enhances VOC caused by the sickled erythrocyte. Lower levels of NO and vasoconstriction may also result from conditions that interfere with NO bioavailability (Marin & Rodriguez-Martinez, 1997) such as elevated levels of cell-free hemoglobin in plasma resulting from hemolysis in SCD. The cell free hemoglobin in plasma rapidly destroys or mops up NO (Kaul & Hebbel, 2000; Reiter *et al.*, 2003) thereby limiting NO bioavailability (Reiter, 2002).

Endothelial cells also play a key role in regulating the local vascular function in part by secreting vasodilator NO and vasoconstrictor endothelin-1 (ET-1) during VOC (Haynes & Webb 1998). Interaction between sickled erythrocytes and endothelium may contribute to the vascular occlusion in SCD via several mechanisms, including further erythrocyte sickling and local vascular instability precipitated by the imbalance of local vasoconstrictors and vasodilators produced by endothelial cells in response to interaction with sickled erythrocytes (Kaul *et al.,* 2000; Belhassen *et al.,* 2001).

CONCLUSIONS

Nitric oxide levels were decreased during VOC and rebound significantly during the immediate post crisis period in SCD patients.

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