Distribution of Haemoglobin phenotype and haemoglobin concentration among children and adults in sickle cell disease patients in Portharcourt Rivers state, Nigeria.

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ABSTRACT

The significantly lower Hb levels in sickle cell disease (SCD) (in children and adults) than that of controls was as expected, however the Hb concentration was found to be slightly higher in vaso-occlusive crises (VOC) than steady state. This research was to determine the distribution of haemoglobin phenotype and haemoglobin concentration among children and adults in sickle cell disease patients in Nigeria. This was a case control study carried out in Port Harcourt, Nigeria. There were three groups of 45 subjects each: SCD patients in steady state, SCD in VOC (which constituted the cases) and normal controls with HbAA. These participants had their blood samples analyzed for full blood count. Results were analyzed with the SPSS version 20. Haemoglobin SC (HbSC) patients were seen only in the VOC group, 2 in children and two in adults. This may be because most steady state SCD patients do not usually come for follow up visits, therefore those with relatively more stable disease may attend clinic visits less. Sickle cell patients can adapt to very low haemoglobin (Hb) levels in the steady state, as observed also in this study for both children and adults. Factors like infection cause a further decrease in Hb, leading to hypoxia, ischaemia and crisis.

Keywords: Sickle cell, haemoglobin, hypoxia, ischaemia and patients.

INTRODUCTION

Sickle cell disease (SCD) is a disorder of great medical importance in Africa because of its endemicity and associated significant disability, morbidity and mortality in African part of the world [1, 2]. It has also achieved worldwide recognition because of the movement of people from the endemic regions to regions with low prevalence rates. Sickle cell disease is a multi-organ disease characterized by sickled red cells, premature destruction of red cells (haemolysis), a susceptibility to infection and recurrent blood vessel obstruction causing tissue ischaemia with infarction [3]. The latter is the underlying pathology of recurrent acute episodes of pain which is the hallmark of the disease. Although much of the pathophysiology of SCD is related to the polymerization of sickle haemoglobin (Hb) within the red cells, studies have shown that other metabolic processes such as inflammation, haemolysis, nitric oxide (NO) deficiency, ischaemia and reperfusion injury with oxidative stress and cell adhesion, all play key roles in this disorder [4].

MATERIALS AND METHODS
Study Area

This study was carried out in Port Harcourt, using patients recruited from the University of Port Harcourt Teaching Hospital (UPTH), the Braithwaite Memorial Specialist hospital (BMSH), St Martin's Hospital, and the Palmers' Hospital. Port Harcourt is in the South-South zone of Nigeria. These hospitals are major hospitals that render services to patients within the state and neighboring states including Akwa Ibom, Bayelsa, Imo and Abia.

STUDY DESIGN

This was a hospital-based case control study.

ETHICAL APPROVAL

This study was approved by the UPTH Research and Ethical Board. An informed consent was obtained from all participants of the study.

STUDY POPULATION

The study was composed of a total of 66 children and 69 adults. Study participants were selected in a systematic manner and were divided into 3 subpopulations.

a) SCD patients in steady state who were recruited from the paediatric- (25 from UPTH and 5 from BMH) and adult- (11 from UPTH and 4 from BMH) haematology outpatient clinics of the participating hospitals

b) SCD patients in Vaso-occlusive crisis recruited through the Accidents and Emergency units and children emergency wards of the participating hospitals. There were 20 children recruited from UPTH and 3 from BMH; 13 adult patients from UPTH, 5 from BMH, 2 from Palmers' and 2 from St. Martin's.

c) Normal healthy adult volunteers attending the blood donor unit in UPTH and healthy children of health care workers.

A questionnaire was filled for participants by the researcher after consent was obtained.

Data in the questionnaire included: participant number, age, gender, Hb phenotype, brief history of any acute illness, the presence of bone pain and characterization (site, severity, character and duration), history of any current medication and the test results of this study.

INCLUSION CRITERIA OF CASES

1. All cases with confirmed SCD by Hb electrophoresis. (AND)
2. Consenting SCD child caregiver and adult SCD patients in steady state, (patients with 2 or more month's history of no crisis or blood transfusion) (OR)
3. SCD patients in vaso-occlusive crisis (patients admitted for severe bone pain crisis).

EXCLUSION CRITERIA OF CASES

1. People with Hb AS phenotype.
2. Subjects with SCD complications, or chronic infections, or chronic inflammatory conditions, (for example, leg ulcers, hypertension).
3. Subjects positive for transfusion-transmissible infections (HIV, hepatitis, and syphilis).
4. Refusal to give consent.
5. Pregnant SCD patients.

CONTROL SELECTION PROCESS

Adult controls who were 18 years and above were recruited from the blood donor clinic. Healthy adults with known HbAA phenotype were selected in a systematic manner. Children used for controls were those less than 18 years who were children of healthcare workers in the participating hospitals. These control individuals were not age- and sex- matched with the SCD patients group, but were healthy individuals with normal haemoglobin profiles and lacked a history of anaemia, inflammatory conditions, and haematological diseases.

SAMPLE SIZE DETERMINATION

Sample size was calculated using the prevalence of SCA (2.9%) in the University of Port Harcourt Teaching Hospital and the formula $N = (Z^2 pq)/d^2$.

N= desired sample size
Z= standard deviation at 1.96 (95% confidence interval)
P= proportion of the target population, estimated to have the particular characteristics (2.9% or 0.029)
q =1-p
d = degree of precision used (0.05)

This gives a minimum sample size of 43.3; approximately 45 for each subgroup of participants.
Each of the subgroups (subjects in VOC, in steady state and normal controls), had a sample size of 45 giving a total of 135.

EQUIPMENT AND MATERIALS
The equipment and materials used are all of analytical standard.

SPECIMEN COLLECTION, PREPARATION AND STORAGE
For every participant, a total of 6mls of venous blood was collected by venepuncture using aseptic techniques.

1) Three millilitres of venous blood was dispensed into ethylenediamine tetra-acetic acid (EDTA) bottles for full blood count (FBC) consisting of haemoglobin, total white cell count and differentials, platelet count and absolute reticulocyte count; and serological screening for hepatitis B and C, syphilis and HIV 1&2. The bottles were filled appropriately to ensure a proper blood to anticoagulant ratio. The samples were mixed immediately by gentle inversion to ensure adequate mixing of blood with the anticoagulant.

2) Another 3mls of venous blood was collected simultaneously and dispensed into serum separator tubes or plain sterile bottles. Clot was allowed to form and the tubes centrifuged at 2500rpm for 10 minutes. [i] Supernatant serum was then transferred to tubes for storage at -20°C.

METHODOLOGY
Full Blood Count was carried out using a 5 part auto analyzer (BC 6800 Autohaematology analyzer system Mindray® product). The samples were placed on the sample mixing machine. The power supply, connections of the analyzer to the reagents or diluents, to the waste and pneumatic unit were checked. The auto-analyzer, loaded with all its reagents for the analysis, and the PC software were switched on and allowed to boot (analyzer automatically performed a self test procedure, background cycle and initialized the system). The control samples were run first. A clean uncapped EDTA tube was presented to the sample probe, making sure the probe goes deep into the bottom of tube to avoid spills, and bubbles. The aspirate button was pressed to start dispensing the diluents; the tube was removed when buzzer sounds. The machine analyzed a sample in 1.5 minutes; the machine automatically displays the results, which is printed from the printer.
Serology: Anticoagulated whole blood was centrifuged at 2500rpm to separate plasma, and serological screening for HIV, hepatitis B & C and VDRL were carried out using the rapid test strips (Determine HIV-1/2, Diaspot HBsAg and ABON Biopharm HCV and Syphilis). Two drops of patients plasma was applied to the sample application area or pad. Test was read after 15 minutes. A positive result was shown by a pink line in both the control and test areas (areas were clearly indicated on strips) and a negative result was shown by a pink line in the control area only.
Hb Phenotype: Hb electrophoresis for Hb phenotype was carried out on all samples. This was determined using cellulose acetate electrophoresis in alkaline buffer. Cellulose acetate strips are gently blotted after soaking in Tris buffer. 2 microlitre of haemolysate (control and test samples) were applied in a line midway between the centre and the cathodic end of each strip. The strips were quickly transferred to the electrophoretic tank. Current was applied and electrophoresis was carried out for about 30 minutes at 220 volts across the strip. At the end of separation, the strips were removed using forceps and dried in a hot air oven. The test bands were inspected and interpreted in relation to the controls

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STATISTICAL ANALYSIS

Data was entered and analyzed using the IBM statistical package for social sciences software (SPSS) version 20. Descriptive statistics (mean, standard deviation, percentages and charts) were used to summarize the variables and characterize the demographics. Student's t- test and Analysis of variance (ANOVA) were used to compare the differences in means between two and three groups respectively. Post hoc test was performed using Scheffe test to explore significant mean differences across groups. Chi Square or Fishers exact tests were used to compare differences in proportions across groups. Pearson's correlation coefficient was used to examine the correlation between TNF, SAA and the haematological parameters. P-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION
DEMOGRAPHICS OF THE STUDY PARTICIPANTS

There was a total of 135 study participants, 66 were children, 53 had sickle cell disease (SCD) with 13 HbAA controls; 37(56.1%) were males and 29(43.9%) were females. Their ages ranged between 4 and 17 years. (Table 1)

There were also 69 adults, 37 SCD and 32 HbAA controls; 41(59.4%) were males and 28(40.6%) were females. Their ages ranged between 18 and 38 years. (Tables 2 and 3)

The VOC subgroup: This group had a total of 45 participants. The 23 children participants were 13(56.5%) males and 10(43.5%) females. They had a mean age of 9 ±3 years (Table 4).

The clinical features in the sickle cell VOC study group

The pain occurred in bones and joints for the patients in VOC in this study, the major sites of pain encountered were the lower limbs (49%), the upper limbs (25%) and chest (25%). Twenty patients (44.4%) in VOC recorded fever, with body temperatures ranging between 37.7- 39^{0}C. Eleven (24.4%) of these patients had cough, 3 (6.7%) had pain on urination (dysuria), and 37 (82.2%) of them had jaundice of varying degrees. All the patients had taken some form of analgesia prior to presentation which included the use of non steroidal anti-inflammatory drugs (NSAIDs) in 45% cases; pentazocine in 21%; tramadol (15%) and paracetamol (15%). All patients and controls in this study were seronegative for HIV, hepatitis B &C and VDRL.

HAEMATOLOGICAL INDICES IN STUDY PARTICIPANTS

The ranges for Hb concentration in the subgroups were 5.9 to 9.4g/dl (VOC); 5.7 to10.5g/dl (steady state); and 11.3 to 16.2g/dl (control). Two children who came in VOC (8.7%)and 2 in steady state (6.7%) had Hb>9g/dl; 6 in VOC(26.1%) had Hb between 8.1-9g/dl compared to 7(23.3%) in the steady state; 13 in VOC (56.7%) with Hb between 6-8g/dl compared to 18 (60%) of the steady state group; and 2 (8.4%) with< 6g/dl against 3 (10%) in the steady state group. The differences in Hb concentration were not found to be statistically significant. The adult group had 6 cases in VOC (27.3%) and 3 in steady state (20%) having Hb >9g/dl; 3 in VOC (13.6%) had Hb between 8.1-9g/dl compared to 4(26.7%) in the steady state; 13 in VOC (59.1%) with Hb between 6-8g/dl compared to 8 (53.3%) of the steady state group. No adult case had Hb < 6g/dl in both VOC and steady state. The differences in Hb concentration between the two groups were not found to be statistically significant.

Significantly lower levels of Hb concentration were found in the SCD groups than control group (*p*- value <0.001), in both children and adults.

The children had a mean Hb concentration for the VOC group as 7.88± 1.37g/dl, this value compared statistically to the steady states mean concentration (conc.) of 7.59± 1.09g/d l, and the control mean of 12.21±0.73 g/dl was statistically significant. Similarly, the adult group had a mean Hb concentration for the VOC group as 8.33± 2.57g/dl, this value compared statistically to the steady states mean concentration (conc.) of 7.91± 1.39g/d l, and the control mean of 13.84±1.37 g/dl was statistically significant.

In the paediatrics group, comparing the mean counts across the three groups showed that the SCD cases had significantly higher reticulocyte count, white cell count, neutrophil count, lymphocyte and eosinophil counts than the control cases.

The adult study group showed significantly higher values in the SCD when compared to the controls in all the parameters, (reticulocyte, white cell, neutrophil, lymphocyte, eosinophil and basophil counts).

Table 1: Age and sex distribution of children among the study groups

Variables	VOC n =23(%)	Steady State n =30(%)	Control n =13(%)	Total n =66(%)
Age (years)				
4-10 years	13 (56.5)	19 (63.3)	6 (46.2)	38 (57.6)
11-17 years	10 (43.5)	11 (36.7)	7 (53.8)	28 (42.4)
Chi square =1.112; p value=0.574				
Sex				
Male	13 (56.5)	15 (50.0)	9 (69.2)	37 (56.1)
Female	10 (43.5)	15 (50.0)	4 (30.8)	29 (43.9)
Chi square =1.365; p value=0.505				

Table 2: Mean age of children among the study groups

	VOC n =23	Steady State n =30	Control n =13
Mean age ± SD	*9.4 ± 3.2*	*9.2 ± 3.6*	*10.8 ± 4.0*

S.D – Standard deviation; F test – 15.400, p value=0.0001*

* Statistically significant

Table 3: Age and sex distribution of adults among the study groups

Variables	VOC n =22 (%)	Steady State n =15 (%)	Control n =32 (%)	Total n =69(%)
Age (years)				
18-24 years	9 (40.9)	11 (73.3)	18 (56.2)	38 (55.1)
25-30 years	13 (59.1)	3 (20.0)	7 (21.9)	23 (33.3)
>31 years	0 (0.0)	1 (6.7)	7 (21.9)	8 (11.6)
Mean age ± SD				
Fisher's exact test = 12.640; p value = 0.008				
Sex				
Male	9 (40.9)	5 (33.3)	27 (84.4)	41 (59.4)
Female	13 (59.1)	10 (66.7)	5 (15.6)	28 (40.6)

Chi Square=15.624; p value = 0.0001*	
*Statistically significant	SD- Standard deviation

Table 4: Mean age of adults among the study groups

	VOC n =22	Steady State n =15	Control n =32
Mean age ± SD	24.1 ± 3.6	24.9 ± 3.5	26.4 ± 6.4

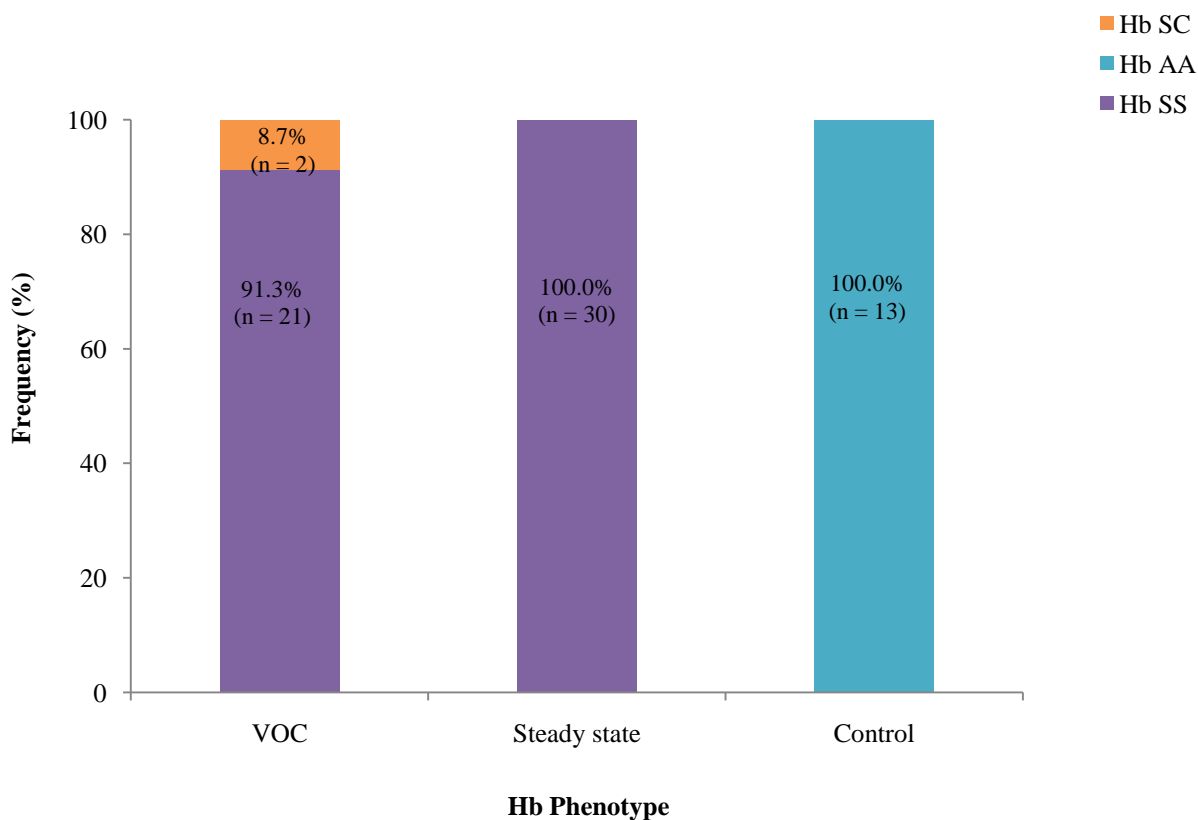S.D – Standard deviation; F test – 2.744, p value=0.072

Figure 1: Distribution of Haemoglobin phenotype among children in the study population
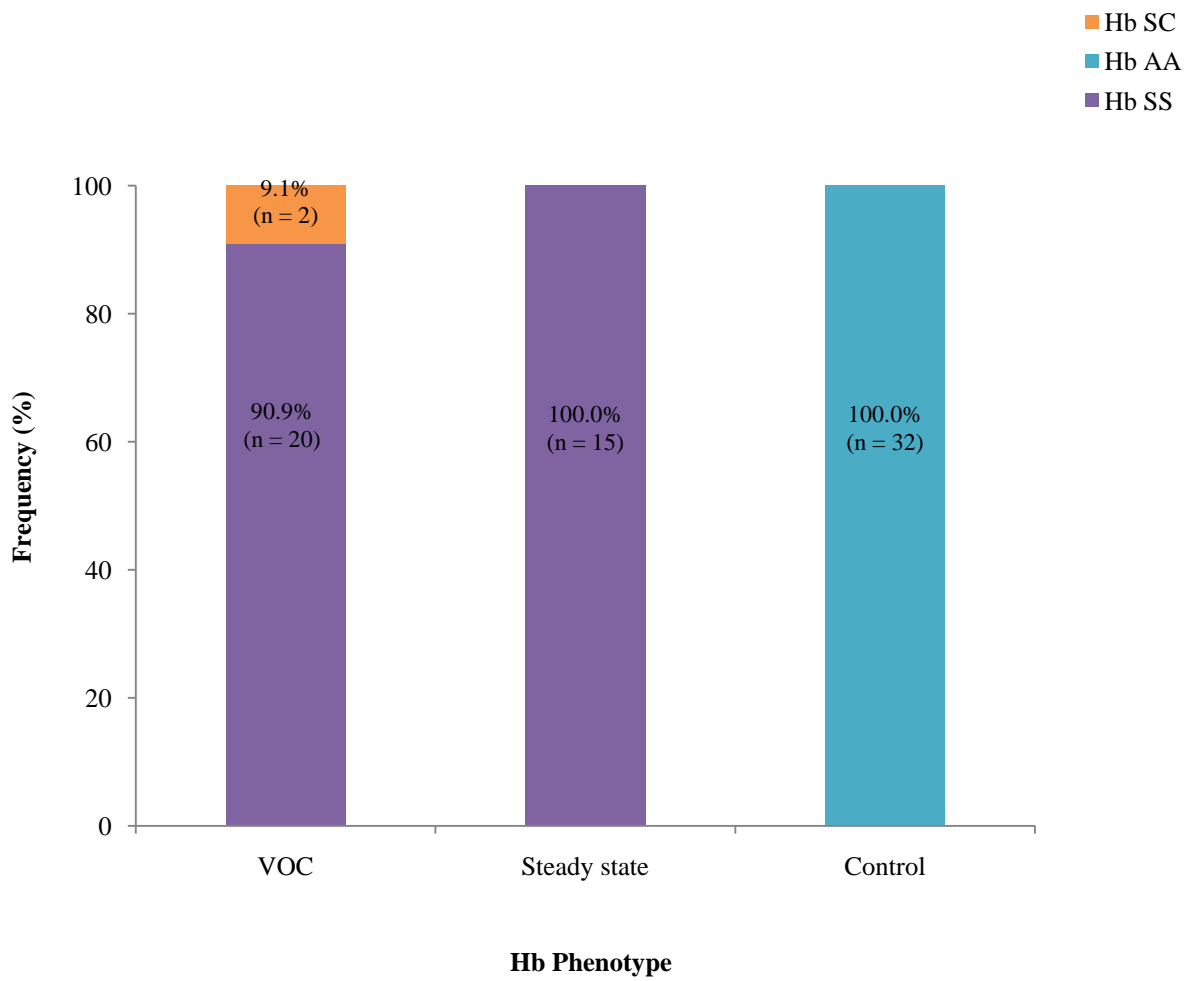
Figure 2: Distribution of Haemoglobin phenotype among adults in the study population

Table 5: Distribution of haemoglobin concentration among children in VOC and Steady state

Haemoglobin concentration	VOC n =23(%)	Steady state n =30(%)	Total n =53(%)
Normal (>9g/dl)	2 (8.7)	2 (6.7)	4 (7.5)
Mild anaemia(8.1-9.0g/dl)	6 (26.1)	7 (23.3)	13 (24.5)
Moderate anaemia(6.0-8.0 g/dl)	13 (56.5)	18 (60.0)	31 (58.5)
Severe Anemia (<6g/dl)	2 (8.7)	3 (10.0)	5 (9.5)
Total	23 (100.0)	30 (100.0)	53 (100.0)

Fisher's exact test = 0.433; p value =1.000

Table 6: Distribution of haemoglobin concentration among adults in VOC and Steady state

Haemoglobin concentration	VOC n =22(%)	Steady state n =15(%)	Total n =37(%)
Normal (>9g/dl)	6 (27.3)	3 (20.0)	9 (24.3)
Mild anaemia (8.1-9.0 g/dl)	3 (13.6)	4 (26.7)	7 (18.9)
Moderate anaemia (6.0-8.0 g/dl)	13 (59.1)	8 (53.3)	21 (56.8)
Severe Anemia (<6g/dl)	0 (0.0)	0 (0.0)	0 (0.0)
Total	22 (100.0)	15 (100.0)	37 (100.0)

Fisher's exact test = 1.094; p value =0.662

DISCUSSION

Haemoglobin SC (HbSC) patients were seen only in the VOC group, 2 in children and two in adults. This may be because most steady state SCD patients do not usually come for follow up visits, therefore those with relatively more stable disease may attend clinic visits less. Also in Nigeria, those with HbSC are found more within the western region, while the study subjects are in the South [4]. There were no other variant sickle haemoglobin observed in this study. It was observed that all the SCD cases in VOC had pre-medicated with analgesia prior presentation. The use of non steroidal anti-inflammatory drugs had the highest frequency (45%), followed by pentazocine (21%), tramadol

and paracetamol at (15%). The major sites of pain manifestation among the SCD cases were in the lower limbs, upper limbs and chest. A minority had pain along the spine and waist. The observed sites of VOC pain agrees with typical findings in children and adults [5, 6].

The control group consisted of 45 participants (13 children and 32 adults). Their mean ages were 11±4 years and 26±6 years respectively. They were not age and sex matched with the SCD patients but they had normal haemoglobin profiles, a negative history of anaemia and inflammatory conditions.

Sickle cell patients can adapt to very low haemoglobin (Hb) levels in the steady state, as observed also in this study for both children and adults. Factors like infection cause a further decrease in Hb, leading to hypoxia, ischaemia and crisis. The significantly lower Hb levels in SCD (in children and adults) than that of controls was as expected, however the Hb concentration was found to be slightly higher in VOC than steady state. This observation was not statistically significant and could be accounted for by dehydration and haemoconcentration at presentation in crisis. The steady state Hb was not significantly different from that of the VOC in both children and adults, thus caution is advised in the transfusion of SCD patients in crisis to avoid overload and hyper viscosity [6].

CONCLUSION

Sickle cell patients can adapt to very low haemoglobin (Hb) levels in the steady state, as observed also in this study for both children and adults. Factors like infection cause a further decrease in Hb, leading to hypoxia, ischaemia and crisis.

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