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Serum levels of copeptin, C-reactive protein and cortisol in different severity groups of sickle cell anaemia

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Summary: It is well known that individuals with SCA undergo constant physiological stress even, in steady state. However, there is little information on the relationship between the severity of sickle cell anaemia (SCA) and serum levels of biomarkers of stress. This study therefore determined the serum levels of copeptin, cortisol and CRP in adults with SCA in different severity groups. Sixty adults with sickle cell anaemia in steady state (27.1±6.3 years) and in vaso-occlusive crisis (24.9±4.9 years) were recruited into this cross-sectional study. Degree of severity (mild, moderate or severe) was determined using a scoring system incorporating annual number of blood transfusions, crisis and presence of anaemia, vaso-occlusive pain and organ complications. Standard methods were used for the determination of packed cell volume (PCV), total white blood cell count (WBC), blood pressure measurements and anthropometric indices. Serum levels of copeptin, cortisol and CRP were determined using ELISA with the ratios calculated accordingly. Data obtained were statistically analyzed using the Student's t-test, Mann Whitney *U* and Chi-square test as appropriate. $P<0.05$ was considered significant. The mean systolic blood pressure (SBP) and copeptin level were significantly higher in subjects with moderate SCA compared with those with mild SCA. Similarly SBP, pulse, WBC, copeptin and cortisol were significantly higher while body weight was significantly lower in subjects with severe SCA compared with subjects with mild SCA. However, WBC and cortisol-to-copeptin ratio were significantly higher in subjects with severe SCA compared with subjects with moderate SCA. There was progressive rise in serum levels of CRP from mild SCA through severe SCA but the differences were not statistically significant. Also, proportions of subjects with elevated SBP and WBC were higher than the proportion of subjects with lower SBP and WBC in the severe SCA group. Serum levels of cortisol, copeptin, and their ratio could differentiate severe SCA from mild or moderate SCA. Also, elevated systolic blood pressure and total white blood cell count are associated with severe sickle cell anaemia.

Keywords*:* Copeptin, Cortisol, Severity score, Sickle cell anaemia, Systolic blood pressure, Vaso-occlusive crisis

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INTRODUCTION

Sickle cell anaemia (SCA) is a genetic disease involving a wide spectrum of disorders. It occurs as a result of substitution of adenine with thymine in the glutamic DNA codon which results in substitution of $ß_6$ valine for glutamic acid (Omoti, 2005; Akinbami et al., 2012).

Sickle cell anaemia (SCA) has variable distribution worldwide with Africa having the highest prevalence. It has been reported that Africa has about 75% of the world's annual figure of 300,000 affected new births with Nigeria responsible for half of this (WHO, 2006; Anie et al., 2010; Makani et al., 2011).

Individuals with SCA have compensated state of ill health (or steady state) interspersed with periods of acute exacerbation characterized as hyperhaemolytic (anaemic) or vaso-occlusive (painful) crisis (Platt et al., 1994). Episodes of vaso-occlusive crisis (VOC) are caused by any factor that significantly increases the rate of sickling and/or decreases the rate of un-sickling to a level that would significantly shift the clinical status of an SCA patients from steady state to crisis (Ahmed, 2011).

Three indices including frequency of crisis, degree of anaemia and the number of organ complications were used to classify Nigerian SCA subjects into mild, moderate and severe groups (Hedo et al., 1993). Subjective criteria such as pain scale, effect of pain on daily activities and the doctors assessment, and the objective criteria such as systolic blood pressure and the respiratory rate have also been used to determine severity in SCA subjects in acute painful crisis (Kotila and Ocheni, 2005).

The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to stress. Measurement of certain hormones such as cortisol produced by the axis has been used to determine degree of stress in various clinical conditions however; this is dependent on the integrity of the axis (Nickel et al., 2012). Cortisol is an established stress hormone which is involved in improvement of hemodynamic status, protection against heightened inflammation and instant provision of energy (Marik and Zaloga, 2002). Osifo et al. (1988) reported that cortisol production is lower in SCA patients but, increases during painful crises.

Copeptin (a 39-amino acid glycosylated peptide) is secreted stoichiometrically from the neurohypophysis. It is produced in equimolar ratio to vasopressin. Copeptin has been shown to be more stable than vasopressin and its level is easy to determine (Katan and Christ-Crain, 2010). Copeptin has been shown to be elevated consequent upon major stress (Nickel et al., 2012). Katan et al. (2008) also reported a gradual increase in copeptin level with increasing level of stress.

C-reactive protein (CRP) is an important acute phase protein usually used as a biomarker for both acute and chronic inflammation (Schultz and Arnold, 1990). Reports have shown that inflammatory markers such as C-reactive protein (CRP) and tumour necrosis factor-alpha (TNF-α) are elevated in SCA patients even in steady state (Raphael and Vichinsky, 2005). Akinlade et al. (2013) reported elevated CRP level but lower levels of copeptin and cortisol in individuals with SCA.

Although chronic inflammation (Mohammed et al., 2010) and hypothalamus-pituitary-adrenal (HPA) axis dysfunction (Osifo et al., 1988) have been reported in SCA subjects, there is need to understand the alterations in serum levels of biomarkers of stress such as copeptin, cortisol and CRP in different severity groups of SCA. This serves as the basis for this study.

MATERIALS AND METHODS

Subjects

Sixty subjects with sickle cell anaemia were recruited from the Haematology Day Care Unit, Department of Haematology, University College hospital, Ibadan, Nigeria. They comprised 30 subjects each in steady state (27.1±6.3 years, 18 males, 12 females) and in vaso-occlusive crisis (24.9±4.9 years, 13 males, 17 females). The subjects were consecutively recruited.

Definition of the steady state

Steady state subjects were those without acute clinical symptoms or crisis for at least three months (Akinlade et al., 2013). This was established by a careful history and complete physical examination.

Definition of vaso-occlusive crisis

Subjects with bone and joint pains or multiple sites of pain, requirement for analgesics and patients considering the episode as typical of crisis which necessitates hospital admission were clinically considered as being in VOC (Omoti, 2005).

Exclusion criteria

Subjects with Hb variants different from HbSS (such as HbSC, HbAS), diabetes mellitus, hypertension, human immunodeficiency virus (HIV), hepatitis, cancer and with established endocrine dysfunctions were excluded from this study. Pregnant and lactating mothers were also excluded from the study.

Calculation of the severity score

The severity score was calculated as previously described by Hedo et al. (1993).
Crisis number(s) per year: 0-1 [0], 2-3 [1], ≥4 [2]
Previous blood transfusion: Yes/No; If yes, how many times per year? 1-2 [1], ≥3 [2]
Any of these complications was scored as Yes (1) and No (0):
Pneumonia: Yes/No; Osteomyelitis: Yes/No; Chest syndrome: Yes/No; Heart Failure: Yes/No; Avascular necrosis of femoral head: Yes/No; Renal Failure: Yes/No; Pigment gallstone & Jaundice: Yes/No; Liver Failure: Yes/No; Seizure: Yes/No; Growth retardation: Yes/No; Dehydrated: Yes/No; Acute Splenic Sequestration: Yes/No; VOC/Pain: Generalised (2), localised(1), No(0). Anaemia: Hb≥10g/dl(0); Hb≥8<10g/dl(1); Hb≥6<8g/dl(2); Hb≥4<6g/dl(3); Hb<4g/dl(4)
The total severity score was calculated as mild SCA (≤3), moderate SCA (>3but≤7) or Severe SCA (>7).

Ethical consideration

This study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee (UI/EC/12/0059). Also, informed consent was obtained from each subject.

Anthropometric and blood pressure measurement

Height (m) was taken using a Stadiometer while body weight (kg) was taken using a body weight weighing scale with the subject wearing light clothing and without shoes. Body mass Index (BMI) was calculated as the ratio of weight (kg) to the square of height (m^2). Blood pressure (BP) was obtained using a Mercury Sphygmomanometer after at least 10 minutes of rest.

Sample collection

About 5 ml of venous blood was obtained from each subject. An aliquot of the sample collected was dispensed into EDTA bottles and was used to determine the genotype, total white blood cell counts (WBC) and packed cell volume (PCV) within 2 hours of collection. The remaining samples were dispensed

into plain bottles to obtain sera which were stored at -20°C until analyzed.

Assay methodology

ELISA was used for the determination of serum levels of copeptin (Glory Biosciences, USA), CRP (Immuno-Biological Laboratories, Inc. USA) and cortisol (Rapid Labs Ltd, UK). The total white blood cell count was determined using the automated analyzer (Coulter SwelabAnalyzer) while haemoglobin genotype was done using haemoglobin electrophoresis at pH 6.8. Microhaematocrit centrifuge was used for the determination of packed cell volume (PCV) as described by Cheesbrough(2000).

Statistical analysis

Copeptin (with Gaussian distribution) was compared between groups using the Student's t-test while CRP and cortisol (with non-Gaussian distribution) were compared using Mann -Whitney *U* test. Chi-Square test (X^2) was used to find relationship between qualitative variables and $P<0.05$ was considered to be statistically significant. The SPSS statistical software program version 17.0 (SPSS Inc, Chicago, IL) was used for the statistical analysis.

RESULTS

The mean SBP was significantly higher in subjects with moderate SCA compared with those with mild SCA. Similarly, SBP, pulse, WBC were significantly higher while body weight was significantly lower in subjects with severe SCA compared with subjects with mild SCA. However, only WBC was significantly higher in subjects with severe SCA compared with subjects with moderate SCA (Table 1).

In Table 2, subjects with moderate SCA had significantly higher levels of copeptin compared with subjects with mild SCA. Significant elevation of cortisol and copeptin was observed in subjects with severe SCA compared with subjects with mild SCA. Cortisol-to-copeptin ratio was however higher in subjects with severe SCA compared with those with moderate SCA. Furthermore, there was progressive rise in serum levels of CRP from mild SCA through severe SCA but the differences were not statistically significant.

Elevated systolic blood pressure (SBP) and total white blood cell count (WBC) were significantly associated with severe SCA. Proportion of subjects with elevated SBP and WBC was higher in the severe SCA group than the proportion of subjects with lower SBP and WBC. In contrast, the proportion of subjects with elevated diastolic blood pressure (DBP) was lower in the severe SCA group than the proportion of subjects with lower DBP (Table 3).

Table 1: Characteristics of the subjects

	Mild (n = 23)	Moderate (n = 20)	Severe (n = 17)
Age (years)	26.83±5.36	25.50±6.35	25.18±5.39
Height (m)	1.66±0.11	1.63±0.10	1.60±0.09
Weight (kg)	58.48±9.75	54.80±10.51	52.59±7.55[a]
BMI (kg/m^2)	21.23±3.37	20.60±2.36	20.41±2.28
Pulse (b/m)	80.00±2.91	83.80±10.83	86.47±8.93[a]
SBP (mmHg)	107.83±6.00	113.50±8.75[a]	118.82±14.53[a]
DBP (mmHg)	68.26±5.76	68.75±8.57	72.94±11.60
PCV (%)	22.84±3.72	24.21±7.23	21.61±4.39
WBC ($10^6/\mu L$)	10.26±3.74	11.92±4.46	15.67±3.93[a,b]

Values are in mean±standarddeviation,[a]significant when compared with mild, [b]significant when compared with the moderate, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, PCV=packed cell volume, WBC=total white blood cell count

Table 2: Copeptin, cortisol, C-reactive protein and their ratio in subjects with mild, moderate and severe sickle cell anaemia

Variables	Mild (n = 23)	Moderate (n = 20)	Severe (n = 17)
Copeptin (ng/ml)	198.13±56.13	259.60±78.58[a]	241.12±78.86[a]
CRP (µg/ml)	20.50 (5.00-66.00)	40.50 (16.75-108.25)	41.00 (15.50-11.50)
Cortisol (nmol/l)	390.00 (360.00-480.00)	470.00 (305.00-655.00)	590.00 (470.00-755.00)[a]
Cortisol-copeptin ratio	2.29±0.77	2.11±1.00	2.79±0.96[b]
Copeptin-CRP ratio	8.39 (3.62-30.91)	7.73 (2.54-13.96)	7.67 (2.09-21.25)
Cortisol-CRP ratio	17.56 (6.88-87.27)	12.88 (5.56-39.47)	17.94 (5.34-48.33)

Values are in mean±standard deviation or median (interquartile range), [a]significant when compared with mild, [b]significant when compared with the moderate, CRP=C-reactive protein

Table 3: Association of sickle cell anaemia severity with age, gender, educational status, occupation, blood pressure, body mass index, packed cell volume and white blood cell count

	Mild & Moderate SCA (n= 43)	Severe SCA (n = 17)	n	X^2	P-value
Age (years)					
<25	19 (67.9%)	9 (32.1%)	28	0.375	0.540
≥25	24 (75.0%)	8 (25.0%)	32		
Gender					
Male	21 (67.7%)	10 (32.3%)	31	0.487	0.485
Female	22 (75.9%)	7 (24.1%)	29		
Educational Status					
Primary & Secondary	9 (69.2%)	4 (30.8%)	13	0.048	0.826
Undergraduate & Graduate	34 (72.3%)	13 (27.7%)	47		
Occupation					
Skilled & intermediate	19 (79.2%)	5 (20.8%)	24	1.205	0.547
Semi-skilled & unskilled	5 (71.4%)	2 (28.6%)	7		
Unemployed	19 (65.5%)	10 (34.5%)	29		
BMI (kg/m^2)					
<18.5	6 (66.7%)	3 (33.3%)	9	0.520	0.771
18.5 – 24.9	32 (71.1%)	13 (28.9%)	45		
≥25	5 (83.3%)	1 (16.7%)	6		
SBP (mmHg)					
<120	33 (82.5%)	7 (17.5%)	40	6.936	0.008*
≥120	10 (50.0%)	10 (50.0%)	20		
DBP (mmHg)					
<80	36 (78.3%)	10 (21.7%)	46	4.222	0.040*
≥80	7 (50.0%)	7 (50.0%)	14		
PCV (%)					
<25	25 (62.5%)	15 (37.5%)	40	3.775	0.052
≥25	15 (88.2%)	2 (11.8%)	17		
WBC($10^6/\mu L$)					
<12	26 (89.7%)	3 (10.3%)	29	8.945	0.003*
≥12	17 (54.8%)	14 (45.2%)	31		

Values are in no of sickle cell anaemia subjects with percentages in parenthesis, X^2= chi-squared test, p = probability, * =significant, n = total number in each group, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, PCV=packed cell volume, WBC=white blood cell count

DISCUSSION

Despite intensive research on sickle cell anaemia, occurrence of severe SCA crisis is still a major health challenge. This highlights the need for further research to reduce SCA morbidity and its attendant complications with a view to preventing vaso-occlusive crisis (VOC), which often predisposes SCA subjects to myriad of complications.

The significant reduction in body weight observed in subjects with severe anaemia compared with mild SCA is in line with the report of Singhal et al. (1993, 1997), Barden et al. (2002) and Akohoue et al. (2007). They reported lower body weight in SCA subjects which was attributed to hypermetabolic state as a result of elevated resting energy expenditure, chronic illness, anaemia, hyperactive erythropoiesis, increased cardiac workload, increased protein turnover, inflammation and oxidative stress. Our observation could indicate that there is poor nutritional status in subjects with severe SCA hence; nutritional need of subjects with severe SCA must always be considered for an optimum management. Subjects with severe SCA are likely to have more complications making them chronically ill with the attendant weight loss.

Blood pressure has been reported to be lower in SCA subjects (Pegelow et al., 1997) but its elevation has been associated with increased risk of stroke and death. Our observed significant elevation of SBP in subjects with severe SCA could indicate progressive increase in the risk of stroke and death as SCA subject progress from mild to severe SCA. DeBaun et al. (2012) reported that higher baseline systolic blood pressure is significantly associated with silent cerebral infarction in subjects with SCA. Our observation could be as a result of reduced bioavailability of nitric oxide (a vasodilator) consequent to possible heightened oxidative stress in subjects with moderate and severe SCA. Bioavailability of nitric oxide (NO) within the vascular wall is limited by superoxide anions in sickle cell anaemia (Ilesanmi, 2010).

The pulse was observed to be significantly higher in subjects with severe SCA compared with subjects with mild SCA. This is not surprising as Covitz et al. (1995) and Sanya et al. (1999) had earlier reported higher resting pulse rate in SCA subjects. This was

attributed to compensatory response to chronic anaemia, which increases cardiac output. Our observation however, indicates that there is progressive rise in pulse rate as SCA progress from mild to severe SCA.

Many complications of SCA have been associated with leucocytosis (Buchanan and Glader, 1978). Akinbami et al. (2012) and Rao et al. (2012) reported elevated WBC in SCA subjects. Our observed elevated WBC in subjects with severe SCA compared with subjects with mild SCA and moderate SCA indicates heightened auto-splenectomy, increased redistribution of the white blood cells between the marginal and circulating pools, higher neutrophil level and anxiety (Milhorat, 1942). Our observation indicates that subjects with severe SCA are more prone to complications associated with leucocytosis.

Cortisol production has been reported to be lower in SCA patients but, increases during painful crisis (Osifo et al., 1988). Although no earlier report was found on the levels of copeptin, CRP and cortisol in SCA subjects in different severity groups, Hedo et al. (1993) reported that IgG and IgG3 subclass levels had significant positive correlation with frequency of crisis and the derived severity score. In this study, copeptin was significantly higher in subjects with moderate SCA compared with those with mild SCA. Similarly, copeptin and cortisol were significantly higher in subjects with severe SCA compared with subjects with mild SCA. Furthermore, cortisol-copeptin ratio was significantly higher in subjects with severe SCA compared with those with moderate SCA. Our observation indicates that there is increased stimulation of the hypothalamo-pituitary-adrenal (HPA) axis as SCA progresses from mild to severe form. Perhaps there is less response to severe stress in the hypothalamus than in the pituitary and adrenal gland or a more exaggerated response distally in the HPA axis than centrally. This indicates that cortisol and copeptin as well as their ratio could be used in assessing the degree of SCA severity. This could be of clinical importance as it circumvents the rigor of determining the multi-indices used in SCA severity score determination. However, a large population study is suggested in order to confirm this observation.

Our observed higher proportion of elevated SBP and WBC in the severe SCA group indicates significant association between severe SCA and elevated SBP and WBC. Our observation indicates that SCA subjects with elevated SBP and WBC are likely to suffer from severe SCA thus, suggesting that monitoring of blood pressure and WBC are important tools in the classification and management of severe SCA.

It could be concluded from this study that copeptin, cortisol and their ratio could differentiate severe SCA from mild or moderate SCA. Also, elevated systolic blood pressure and total white blood cell count are associated with severe sickle cell anaemia.

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