

Full Length Research Paper

# Serum cardiac troponin T (cTnT) in Nigerian children with sickle cell anaemia: an index of myocardial injury?

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Recurrent vaso-occlusion in sickle cell anaemia (SCA) may be associated with myocardial ischaemia. We determined the serum cardiac Troponin T (cTnT) profile in children with SCA and assessed its relationship with the vaso-occlusive crises pain severity scores. Serum Troponin levels of 26 children with SCA who had significant painful crisis (cases) and 27 SCA in stable state (controls) were determined using microparticle enzyme immunoassay method. The severity of the pain among the cases was determined using age appropriate pain rating scales. The overall mean (Standard deviations, SD) serum cTnT for the 53 children was 65.63(23.54) ng/L with 45.3% having normal serum levels. Cases however had significantly higher mean serum cTnT than the controls, 119(24.40) vs. 57.90(13.80) ng/L,  $p=0.001$ . Also, significantly higher proportion of the cases, 19 (73.1%), than the controls, 3 (11.1%) had elevated serum cTnT levels (i.e. serum levels  $>100$  ng/ L),  $p=0.001$ . Serum cTnT levels had strong positive correlation with the pain severity scores; Pearson's correlation coefficient ( $r$ ) =0.64,  $p=0.011$ . Routine serum cTnT estimation may be useful in evaluating for subclinical myocardial ischemic damage which may be associated with vaso-occlusive crisis in children with SCA.

**Key words:** Infarction, ischaemia, pain rating scale, troponin, vaso-occlusive crisis.

## INTRODUCTION

Sickle cell anaemia (SCA), the most severe form of sickle cell disease is characterised by a single  $\beta$  globin sickle mutation. It manifests with several acute medical emergencies including stroke, kidney dysfunction, acute chest syndrome and acute hepatic/ splenic sequestration among others (Weatherall and Clegg, 2001). Diagnosis of myocardial infarction (MI) is not commonly made in children with sickle cell disease, even though the disease is often characterised by recurrent painful episodes of body parts which results from vaso-occlusion. Physicians tend to believe that the heart is not an organ of high risk for vaso-occlusion and infarction in children (de Montalembert et al., 2004), plausibly because infarctions of the heart are often clinically overshadowed by severe musculo-skeletal pains (Bode-Thomas et al., 2011; Mansi and Rosner, 2002). Also, children have lower serum

cholesterol (Mansi and Rosner, 2002). In adults, however, because of the repeated infarctions of the heart and occlusion of its microvasculature together with higher serum levels of cholesterol, left ventricular dysfunction, chronic heart failure and MI are encountered in 10 to 30% of the adult population (Haywood, 2009).

Cardiac enzymes are released into the bloodstream during myocardial damage and these can be used in the diagnosis of MI (Mansi and Rosner, 2002). Although, there are many cardiac enzymes whose serum levels are elevated following myocardial injury, cardiac Troponins are the most sensitive and specific biomarkers of myocardial injury than other markers (90 to 94% sensitive and about 95% specific for myocardial injuries). They are intracellular markers released during episode of myocardial injury (Mansi and Rosner, 2002; Space et al., 2000). Others, such as Glutamic Oxaloacetic Transaminase (SGOT) also called Aspartate Aminotransferase (AST); Lactate Dehydrogenase (LDH) and Creatine Phosphokinase (CPK) are also elevated in

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**Table 1.** Face, legs, activity, cry, consolability (flacc) pain rating scale for young children ( $\leq 2$  years).<sup>12</sup>

Categories/ Score	0	1	2
Face	No particular expression or smile	Occasional grimace or frown, withdrawn or disinterested	Frequent-to-constant quivering chin, clenched jaw
Legs	Normal position or relaxed	Uneasy, restless or tense	Kicking or legs drawn up
Activity	Lying quietly, normal position, moves easily	Squirming, shifting back & forth, tense	Arched, rigid or jerking
Cry	No cry (awake or asleep)	Moans or whimpers; occasional complaints	Crying steadily, screams or sobs, frequent complaints
Consolability	Content, relaxed	Reassure by occasional touching, hugging or being talked to distractible	Difficult to console or comfort

**NB:** Each of the 5 categories is scored from 0 – 2 resulting in a total score of 0 – 10; Significant pain  $\geq 4/10$

some other conditions such as trauma to the skeletal muscles, in liver disease, pancreatitis, intramuscular injection, cerebral vascular disease and strenuous exercise (Haywood, 2009). The fact that these enzymes are also found in other tissues, therefore makes reliance on them as biomarkers of MI unsure. Evaluation of serum cardiac Troponin on the other hand may provide information on the possibilities of myocardial injury in children with sickle cell anaemia.

There are two major subtypes of cardiac troponin – Troponin T (cTnT) and Troponin I (cTnI). Although cTnT are more readily measured, both have equal sensitivity and specificity to cardiac injury (Space et al., 2000). They regulate contractility of the heart by controlling interactions of actin and myosin in the heart muscles. Normal levels of cardiac troponin in the blood are very low ( $< 100\text{ng/L}$  for cTnT and  $< 400\text{ ng/L}$  for cTnI), but they rise sharply and quickly in response to a heart muscle injury and are therefore valuable at detecting heart injury early, even if very mild (Space et al., 2000).

With improvement in Sickle Cell Disease (SCD) care and subsequent survival, cardiovascular abnormalities including ischaemic heart changes are likely to be on the increase. This study was therefore conducted to assay the serum levels of cTnT (a very sensitive and specific cardiac biomarker) in children with SCA. We also assessed the relationship between this enzyme and the severity of pain.

## METHODS

Children with SCA who presented in the Children's Emergency Ward of the Wesley Guild Hospital Unit of Obafemi Awolowo University Teaching Hospital, Ilesa with significant painful crisis over nine-month period of

the study (1st February to 31st October, 2012) were recruited. Age- and sex-matched children with SCA in stable state who were attending the Paediatric Haematology Clinic of the hospital for routine follow up care during the period were also recruited as controls. A child was said to be in stable state when he/ she is free of crisis and infection for at least four consecutive weeks after a previous crisis and three months after the last blood transfusion (Ballas et al., 2000). Significant painful episode was taken as an acute painful event requiring treatment at a healthcare facility or at home with either (a) parenteral or an equianalgesic dose of oral narcotics or (b) parenteral or an equianalgesic dose of oral non-steroidal anti-inflammatory drugs (Edwards et al., 2005; Serjeant, 1988).

Children with underlying cardiac disease (based on previous record and or echocardiogram report), elevated blood pressure, electrolyte derangement, renal failure, under-nutrition or those receiving anti-arrhythmic drugs such as digoxin were not included in the study. The cases were managed with generous hydration, analgesics, supplemental oxygen, antimalaria, antibiotics and blood transfusion. Those in stable state received routine haematinics (Folic acid and Vitamin B complex) and prophylactic antimalarial chemotherapy (Proguanil). None of the children was on chronic blood transfusion programme or hydroxyurea at the time of the study. A written informed consent was obtained from the parent(s)/ caregiver(s) before recruitment.

A structured questionnaire was used to obtain information on each child's sociodemographic data and the present clinical profile. The socioeconomic class of the parent was based on the highest educational attainment and the occupation of the parents as described by Oyedeji (Oyedeji, 1985). Detailed clinical examination findings were also recorded. For the cases,

**Table 2.** Sociodemographic characteristics of the subjects.

Sociodemographic characteristics	Cases	Controls	Total	$\chi^2 / t$	P
	N = 26 n (%)	N = 27 n (%)	N = 53 n (%)		
Males	17 (65.4)	19 (70.4)	36 (67.9)	0.151	0.697
Females	9 (34.6)	8 (29.6)	17 (32.1)	0.151	0.697
Infants	2 (7.7)	3 (11.1)	5 (9.4)	0.000	1.000 <sup>*</sup>
1 – 5 years	12 (46.2)	6 (22.2%)	18 (34.0)	3.382	0.066
6 – 10 years	9 (34.6)	13 (48.1)	22 (41.5)	0.999	0.318
> 10 years	3 (11.5)	5 (18.5)	8 (15.1)	0.106	0.745 <sup>*</sup>
Mean (SD) age [years]	6.30 (1.69)	7.18 (4.38)	6.16 (3.52)	0.96	0.343 <sup>#</sup>
Upper SEC (I and II)	9 (34.6)	11 (40.7)	20 (37.7)	0.212	0.646
Middle SEC (III)	9 (34.6)	8 (29.6)	17 (32.1)	0.151	0.811
Lower SEC (IV and V)	8 (30.8)	8 (29.6)	16 (30.2)	0.008	0.928

\*Yates corrections applied; # Independent t test was applied; SEC = Socioeconomic class.

severity of painful crisis was assessed with Pain Rating Scale (PRS). Numerical Pain Rating Scale (N-PRS) was used for children older than 5 years, as long as they can count and have some concept of numbers and their values in relation to other numbers. This scale uses a straight line with end points identified as “no pain” and “worst pain”; divisions along line are marked in units from 0 to 10 corresponding to pain severity score. Wong-Baker faces Pain Rating Scale (WB-PRS) was employed for children between three and five years. This scale was designed by Whaley and Wong in 1987 (Whaley and Wong, 1987). It consists of six cartoon faces ranging from a smiling face for “no pain” to tearful face for “worst pain” (scores range from 0 to 10, with 0 for no pain and 10 for the worst pain). For younger children  $\leq$  two years, expression on the face, positioning of the legs, Activity, Cry, Consolability (FLACC) (Table 1) was used (Voepel-Lewis *et al*, 2010)

### Laboratory investigations

Five milliliters of peripheral venous blood was obtained from each patient at presentation. The sample was kept in a cool place away from light to clot and retract after which the serum was obtained by centrifugation at 3000 revolutions per minute for 5 minutes using a clinical macro centrifuge (Hettich Universal 11, England). The serum was then frozen at  $-20^{\circ}\text{C}$  and transported ice-packed to the Chemical Pathology Laboratory at the Obafemi Awolowo University, Ile-Ife, Nigeria for serum cTnT analysis. The analysis was done by microparticle enzyme immunoassay (MEIA) with fluorometric detection

of enzyme-hydrolyzed fluorescent product (Balas *et al*, 2010).

### Statistical analysis

Data were analysed using SPSS version 17.0. The baseline characteristics of the cases and the controls were compared using Chi-square tests of association and independent sample t-test, with application of Yates correction as indicated. The mean serum levels of cTnT of the two groups were compared using independent sample t-test. The relationship between serum cTnT and pain severity score was analysed using Pearson's correlation coefficient. *P*-values less than 0.05 were considered statistically significant.

## RESULTS

Fifty-three children (26 cases and 27 controls) were recruited for the study. The overall mean (SD) age was 6.16 (3.52) years (range = 6 months to 15 years) with male to female ratio of 2.1: 1 and they were almost equally distributed in the three socioeconomic classes. As shown in Table 2, the two groups (cases and the controls) had similar sociodemographic characteristics.

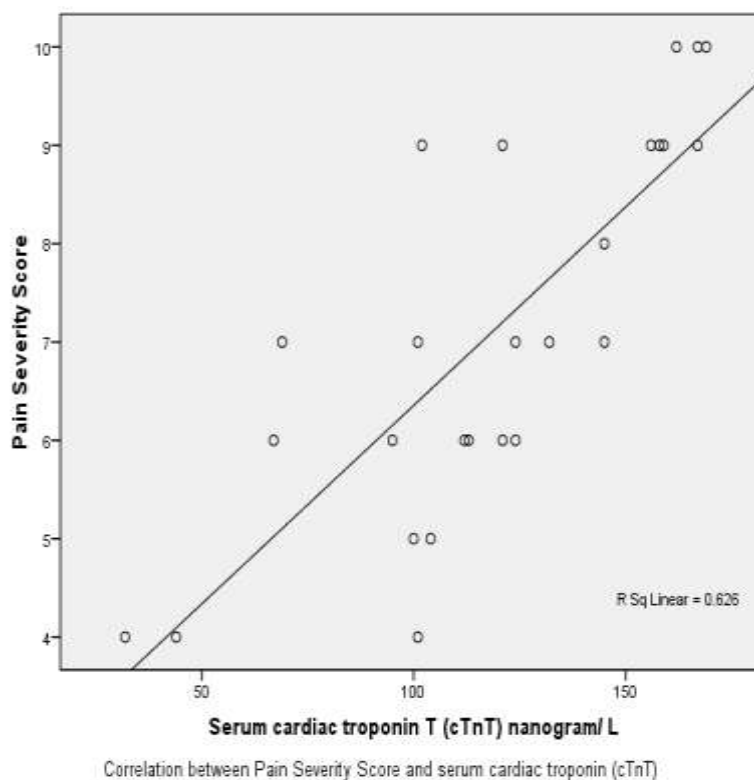
### Serum Troponin T levels

The overall mean (SD) serum cTnT for the 53 children was 65.63 (23.54) ng/L. Twenty-four (45.3%) of them

**Table 3.** Serum cTnT levels of the cases and the controls.

Serum Troponin T (ng/ L)	Cases N = 26 n (%)	Controls N = 27 n (%)	Total N = 53 n (%)	$\chi^2 / t$	P
Low [ $<50$ ]	2 (7.7)	5 (18.5)	7 (13.2)	0.575	0.448*
Normal [50–100]	5 (19.2)	19 (70.4)	24 (45.3)	13.980	0.001
Elevated [ $> 100$ ]	19 (73.1)	3 (11.1)	22 (41.5)	18.473	0.001*
Mean (SD)	119 (24.40)	57.90 (13.80)	65.63 (23.54)	11.28	0.001#
Range	32.20 – 169	15.50 – 84.50	15.50 – 169		

\*Yates corrections applied; # Independent t test applied

**Figure 1.** Correlation between Pain Severity Score and serum cardiac Troponin T level, cTnT.

had normal serum cTnT levels. Cases (SCA with significant pain crisis) however had significantly higher mean serum cTnT than the controls (SCA in stable state), 119 (24.40) vs. 57.90 (13.80) ng/ L,  $p = 0.001$  (Table 3). Also, significantly higher proportion of the cases, 19 (73.1%), than the controls, 3 (11.1%) had elevated levels of serum cTnT (i.e. serum levels  $>100$ ng/ L),  $p = 0.001$ .

#### Pain severity score and serum cTnT levels.

Among the cases, the pain severity score (PSS) ranged from 4 to 10 with a mean (SD) of 6.9 (1.5). Two-third of

them had moderate pain (PSS of 4 to 7), while a third had severe pain (PSS  $>7$ ). Using Pearson correlation analysis, serum cTnT had strong positive correlation with the pain severity score as shown in figure 1. The Pearson's correlation coefficient ( $r$ ) was 0.64,  $p = 0.011$ .

#### DISCUSSION

Although few other studies (de Montalembert *et al*, 2004; Bode-Thomas *et al*, 2011, Mansi and Rosner, 2002) have tried to demonstrate presence of myocardial infarctions in children with sickle cell anaemia using echocardiogram

and/ or electrocardiogram, our study is the first in sub-Saharan Africa to describe the profile of serum cTnT, an enzyme that is very specific for heart injury in this group of children. Children with SCA who presented with painful crisis had significantly higher mean serum level of the cardiac troponin T than their counterparts in the steady state. Since the two groups did not differ in their baseline sociodemographic characteristics, the most plausible explanation for this higher level of the enzyme was the vasoocclusion of microvasculature including those of the heart. The level of this enzyme may therefore help in identifying SCA children with various degree of myocardial injuries.

Due to recurrent vasoocclusion and the resultant ischaemic changes in the organs including myocardium, clinically evident myocardial infarction has been reported in adolescents and adults (Uzsoy, 1964; Reimer and Jennings, 1986). Also, researchers have demonstrated patchy and microscopic fibrosis in the myocardium of children with SCA during autopsy. Although similar report in younger children with SCA is lacking, this does not imply that they do not experience microscopic infarctions of the myocardium with repeated vasoocclusion.

Electrocardiogram (ECG) and echocardiogram (ECHO) may have helped provide further substantive evidence of myocardial ischaemia in children with SCA; however, these tools are not readily available for routine evaluation of children in resource-poor settings such as ours. Yet, the corollary is that it is in such settings in Africa that SCA abound. In addition, cTnT will identify more cases of subclinical myocardial ischemia which ECG and ECHO may miss. Perhaps, the various long-term electrocardiographic abnormalities such as arrhythmias, conduction defects and re-polarisation abnormalities like ST-T wave changes and prolonged QT interval (Bode-Thomas *et al*, 2011; Park and Guntheroth, 1992; Bode-Thomas *et al* 2003a,b) observed among children with SCA are plausibly due to repeated subtle and sub-clinical myocardial ischemia. Whereas cardiac imaging often confirms a diagnosis, simpler and less expensive cardiac biomarker measurements should be an initial testing strategy especially for patients at low risk of cardiac death and a step toward making a diagnosis of subclinical cardiac injury.

Expectedly, the severity or degree of pain among our patients (cases) had strong positive correlation with serum cTnT. This suggests that with each painful episode a child with SCA experience, some degree of myocardial injuries may occur, although in majority of cases, these injuries will be clinically non-evident or may be overshadowed by the more visible bone pains. A very sensitive measurement of myocardial injury is highly desirable since cardiac arrest and death may eventually result from severe infarctions and ischaemia of the heart. Regular estimation of serum cTnT level in children with SCA particularly when presenting with significant painful crisis may be a simple life-saving screening tool for

myocardial injuries. Those with elevated serum Troponin levels may benefit from agents such as aspirin. Apart from occlusion of microvasculature by the sickled red blood cells, platelet adhesion, activation and aggregation also contribute to infarction in SCA. Aspirin which have potent antiplatelet activities may therefore prove beneficial in the primary and secondary prevention of myocardial and even coronary artery disease in this group of children.

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