Original Article

# Hypocalcemia and Vaso-Occlusive Painful Crises in Pediatric Sickle Cell Anaemia

**Anthony Chibueze Nlemadim[1], Henry Chima Okpara[2], Chigozie Ikechukwu Uzomba[1], Maxwell Udo Anah[1], Martin Madu Meremikwu[1]**

[1]Department of Paediatrics, University of Calabar Teaching Hospital, Calabar, Cross-River State, [2]Department of Chemical Pathology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

## Abstract

**Background:** Low serum calcium (hypocalcemia) occurs in sickle cell anaemic (SCA) children. Concomitant presence of prolonged corrected QT (QTc) interval on electrocardiogram can ascertain hypocalcemia, especially during vaso-occlusive painful crises (VOC). **Aim:** The aim of the study was to determine the relationship between hypocalcemia and VOC. **Methods:** It was a prospective cohort study of 38 SCA children aged 4–17 years during VOC and when the same children were in steady state. Information regarding bone pain and clinical examination were obtained, following which electrocardiography was done during both clinical states. Blood was drawn for total calcium and serum albumin estimation. **Results:** The mean (standard deviation [SD]) of total calcium was significantly lower during VOC (1.90 [0.19] mmol/l) than during follow-up steady state (2.24 [0.22] mmol/l), $P < 0.001$. The mean rank of QTc interval was significantly higher during VOC (19.93) than in follow-up steady state (14.50), $P < 0.001$. Total calcium negatively correlated with QTc intervals during VOC ($r_s[36] = -0.36$, $P = 0.029$) and follow-up steady state ($r_s[36] = -0.49$, $P = 0.002$), while QTc interval was highly predictive of hypocalcemia (area under the curve [AUC] = 0.82, $P < 0.001$). Similarly, total calcium was highly predictive of VOC (AUC = 0.89, $P < 0.001$) at cutoff point of 2.13 mmol/l with 89.5% sensitivity and 81.6% specificity. Hypocalcemia was significantly observed during VOC than follow-up steady state (89.5% vs. 21.1%, $P < 0.001$) with odds ratio of 21.28 (95% confidence interval: 0.012–0.189; $P < 0.001$). **Conclusion:** Total calcium <2.13 mmol/l is associated with VOC. Regular total calcium tests should be done. SCA children may benefit from routine oral calcium to reduce frequency of VOC.

**Keywords:** Calcium, corrected QT interval, follow-up steady state, hypocalcemia, painful crises, sickle cell anemic children

## Introduction

Sickle cell anemia is a severe form of hemoglobinopathy whereby an individual inherits two hemoglobin S (HbS) which arises as a result of a point mutation in the β-globin gene.[1] This anomaly results in polymerization of HbS and subsequent sickling of the erythrocytes in certain adverse conditions such as infection, inflammation, and dehydration. These conditions initiate cascade of mechanisms, one of which is red blood cell dehydration, that lead to sickling.[1] Thus, sickle cell anaemic (SCA) children present with recurrent acute episodic events most common of which is vaso-occlusive painful crises (VOC).[2] Meanwhile, maintenance of HbS red cell hydration status depends on cation homeostasis, including calcium across its membrane.[3]

Litosch and Lee[4] have shown that calcium permeates HbS erythrocyte membrane and accumulates in the cytoplasm. This accumulation is a result of increased affinity of calcium to the membrane and not merely dysfunctional $Ca^{2+}$-$Mg^{2+}$ ATPase pump.[4,5] In addition, Hänggi *et al.*[6] showed that increased number and activity of the nonselective red blood cell membrane N-methyl D-aspartate receptors, especially during crises, contribute to calcium accumulation in the erythrocytes. Furthermore, during the deoxygenated state as occurs in VOC and which is usually associated with acidosis, calcium accumulates more in the HbS red cells because of increased cellular permeability.[7] This accumulated calcium leads to the activation of the Gardos channel ($Ca^{2+}$ mediated potassium

**Address for correspondence:** Dr. Anthony Chibueze Nlemadim, Department of Paediatrics, University of Calabar Teaching Hospital, PMB 1278, Calabar, Cross-River State, Nigeria.
E-mail: tonynlemas@gmail.com

Access this article online

Quick Response Code:

Website: www.njmonline.org

DOI: 10.4103/NJM.NJM_154_20



**How to cite this article:** Nlemadim AC, Okpara HC, Uzomba CI, Anah MU, Meremikwu MM. Hypocalcemia and vaso-occlusive painful crises in pediatric sickle cell anaemia. Niger J Med 2021;30:34-9.

**Submitted:** 20-Aug-2020 **Revised:** 29-Sep-2020
**Accepted:** 17-Dec-2020 **Published:** 15-Feb-2021

pump) which moves out potassium and water out of the cell, causing cellular dehydration and thus HbS red cell sickling.[3] This intracellular accumulation of calcium in HbS erythrocytes may partly explain the hypocalcemia (low levels of serum calcium) found in sickle cell anemia.

Hypocalcemia and low serum Vitamin D have been found in sickle cell anemia, especially during painful episodes.[8,9] Hypocalcemia has also been linked to reduced dietary intake in SCA children making it a possible factor for frequent VOC.[10] Furthermore, hypocalcemia prolongs ST-segment duration and, as a result, prolongs the corrected QT (QTc) interval.[11] This prolonged QTc has been found in SCA children during VOC when reversible myocardial ischemia occurs.[12,13] This may result in ventricular arrhythmia and cardiac arrest.[14]

Due to the calcium pump variability in HbS erythrocytes, not all cells exhibit increased calcium permeability.[15] This means that low serum calcium can occur as a result of other causes like poor oral calcium intake; in the absence of intracellular calcium accumulation. However, cellular calcium accumulation as a result of ischemic-induced late sodium current in the myocytes during VOC leads to delayed ventricular repolarisation and QTc prolongation.[16] Hence, electrocardiogram (ECG) may be used to confirm the substantial reduction of serum calcium. Therefore, this study aimed to find the relationship between hypocalcemia and VOC among SCA children during VOC and when the same children were followed up during steady state.

## Methods

This was a prospective cohort study which compared serum calcium levels among 38 SCA children during VOC and follow-up steady state. They presented at the Children Emergency room of University of Calabar Teaching Hospital (UCTH) and those who met the inclusion criteria were consecutively recruited from October 2017 to March 2018. Usually, the emergency room sees about three episodes of VOC per week.

### Inclusion criteria

This included SCA children with VOC (bone pain). VOC is defined as a new onset of pain that lasts at least 4 h, for which there is no explanation other than vaso-occlusion, which requires therapy with parenteral opioids in a medical setting.[17] Steady state refers to the "point in time when the patient is not experiencing any acute painful crisis with no previous painful episode for at least four consecutive weeks and no blood transfusion in the previous four months."[18]

### Exclusion criteria

This included presence of symptoms and signs of renal disease, fever, findings of severe malnutrition (body mass index <−3 standard deviation [SD]), severe hypertension, severe anaemia (packed cell volume <16%), cardiac disease, heart failure, or shock because of their association with myocardial ischemia which prolongs QTc interval.[19] Others included intake of drugs such as digoxin, frusemide, captopril, or chemotherapy, presenting with non-VOC acute events, hypokalemia, hyperkalemia, and low glomerular filtration rate for age and sex.

No subject was receiving chronic blood transfusion, hydroxyurea, calcium, or Vitamin D supplementation. Information obtained on each SCA child's history; physical examination including carpopedal spasm, Trousseau sign, and Chvostek sign; ECG; and total calcium level were entered into an interviewer-administered questionnaire.

### Electrocardiography

This was done following pain relief with a dose of intramuscular pentazocine. This was to keep the child stable in order for the electrodes to be applied and allow for polarization of the ECG voltages before recording with GE Marquette Hellige MAC® 1200ST ECG machine, 2009, with part no. 10116831 and serial number 550047690. ECG interpretation followed pediatric standards.[11] QT interval was read while Bazett's formula was used in calculating each subject's heart rate-QTc interval. QTc interval >0.440 s was taken to be prolonged.[11]

### Laboratory tests

Blood sample (4 mL) was drawn from each SCA child without tourniquet, on presentation and six weeks during followed-up visit. The blood was put into plain tubes, allowed to clot, and centrifuged at 3000 revolutions/min for 5 min to obtain sera. A small volume of sera was analyzed for potassium and creatinine using ion-selective electrode and modified Jaffe kinetic method, respectively. Subjects with normal potassium, serum creatinine values for age, and glomerular filtration rate for age and sex were enrolled. The sera were stored in a refrigerator at −20°C for a maximum of 2 weeks before batch analysis for serum calcium and albumin. Total calcium estimation was done by the cresolphthalein complexone method, while albumin estimation was done by the Bromocresol green method. Albumin-adjusted calcium values were calculated using the Payne formula.[20] However, values of total calcium obtained after analysis were the same as those obtained after calculating albumin-adjusted calcium. Total calcium <2.15 mmol/l was defined as hypocalcemia.[21]

Upon admission and after collection of blood and data which lasted <30 min for each subject, intravenous fluid and analgesics, among other treatment, were commenced. After recovery from VOC, each parent was counseled and discharged with hydroxyurea added to their routine drugs.

### Statistical analysis

Normality tests were carried out on data extracted from the questionnaires. Median values of skewed data were presented, while a comparison of QTc interval was done using Wilcoxon signed-rank test. A paired *t*-test was used in the comparison of mean total calcium levels. Spearman rank correlation was used to determine the relationship between total calcium levels and QTc intervals. Receiver operating characteristic (ROC) curve analysis was used to calculate the predictability of total calcium and QTc interval. Categorical variables were

compared using Pearson Chi-square, while binary logistic regression was used to determine the likely occurrence of VOC. Statistical significance was set at $P < 0.05$ at 95% confidence interval (CI).

### Ethics

Ethical clearance was obtained from the Health Research Ethical Committee of UCTH to carry out the study. Written informed consent from the parents or guardian and verbal assent from children above seven years of age were obtained.

## Results

Age, total calcium, albumin, and albumin-adjusted calcium variables were approximately normally distributed, while potassium and QTc interval data were skewed. A total of 38 different children, each with an episode of VOC, were enrolled over six months which is about two episodes of VOC per week. No subject presented with VOC more than once during this period. There was no death. The mean (SD) age of the subjects was 10.6 (3.8) years with a male: female ratio of 1.7:1 and age range of 4–17 years. Majority (78.9%) were of the high social class group. The serum potassium levels were normal with median (interquartile range) values of 4.8 (4.5–5.0) mmol/l and 4.4 (4.0–4.8) mmol/l during clinical states of VOC and follow-up steady state, respectively. Serum albumin levels were also normal with a mean (SD) of 43.5 (5.3) g/l and 43.0 (4.9) g/l during painful crises and follow-up steady state, respectively, and the difference was not statistically significant ($P = 0.608$). No clinical feature of hypocalcemia was observed in any subject.

### Calcium levels and corrected QT intervals

The sum of mean (SD) total and albumin-adjusted calcium was 2.09 (0.28) mmol/l and 2.07 (0.28) mmol/l, respectively. The differences between their values obtained during both the clinical states were statistically significant ($P < 0.001$).

The median (interquartile range) of QTc intervals during VOC and follow-up steady state was 0.447 (0.438–0.459) s and 0.435 (0.417–0.440) s, respectively. The mean rank was significantly higher during VOC [Table 1].

Total calcium had significant but weak negative correlation with QTc intervals during VOC ($r_s[36] = -0.36$, $P = 0.029$) and follow-up steady state ($r_s[36] = -0.49$, $P = 0.002$) [Figure 1]. Similar correlation was observed between albumin-adjusted calcium and QTc interval during VOC ($r_s[36] = -0.39$, $P = 0.015$) and follow-up steady state ($r_s[36] = -0.51$, $P = 0.001$).

### Hypocalcemia and prolonged corrected QT interval

During VOC and follow-up steady state, 89.5% and 21.1% had hypocalcemia, while 68.4% and 21.1% had prolonged QTc interval, respectively. Their relationships were statistically significant [Table 2]. The same findings were observed between prolonged QTc interval and hypocalcemia derived from albumin-adjusted calcium.

Binary logistic regression was performed to ascertain the effects of total calcium and QTc interval in the prediction of the clinical state of VOC. The logistic regression model was statistically significant, $\chi^2$ (2) = 41.719, $P < 0.001$; explained 56.3% (Nagelkerke $R^2$) of the variance in the clinical states and correctly classified 84.2% of cases. Hypocalcemia was more likely to occur among SCA children during VOC with odds ratio of 21.28 (95% CI: 0.012–0.189; $P < 0.001$). Prolonged QTc interval did not significantly predict VOC [Table 3].

However, ROC curve analysis of QTc interval showed that it was highly predictive of hypocalcemia (area under the curve [AUC] = 0.82, $P < 0.001$) with cutoff point of 0.440 s, 71.2% sensitivity, and 88.2% specificity [Figure 2].

Furthermore, ROC curve analysis of total calcium showed that it was highly predictive of the clinical state of VOC (AUC = 0.89, $P < 0.001$) with cutoff point of 2.13 mmol/l, sensitivity of 89.5%, and specificity of 81.6% [Figure 3].

## Discussion

This study has found that total calcium levels were lower during VOC than in follow-up steady state contrary to the QTc intervals which were higher during VOC. Total calcium significantly correlated with QTc interval which was highly predictive of hypocalcemia. Majority of the subjects had hypocalcemia and prolonged QTc interval during VOC than in the steady state while hypocalcemia significantly predicted VOC.

The mean total calcium among the SCA children during both VOC and follow-up steady state was 2.09 mmol/l. This is below the calcium reference interval of 2.15–2.55 mmol/l.[21] This may be due to reduced oral intake of calcium or increased demand due to child growth. Kawchak *et al*.[10] have demonstrated a decline in calcium intake in children as their ages increase. This finding is similar to the work by Oladipo *et al*.[8] where total calcium level was noted to be 2.10 mmol/l among SCA children.

In this study, total calcium serum levels were significantly lower during VOC than in the follow-up steady state. This

**Table 1: QTc interval, calcium, and clinical state of the subjects ($n$=38)**

| Variables | Clinical state | | Test statistic | *P*-value |
|---|---|---|---|---|
| | VOC | Follow-up steady state | | |
| QTc interval (sec) *n* (mean rank) | 35 (19.93) | 3 (14.50) | $T$=−4.74 | <0.001* |
| Total calcium (mmol/l) mean (SD) | 1.90 (0.19) | 2.24 (0.22) | $t$=−10.20 | <0.001* |
| Albumin-adjusted calcium (mmol/l) mean (SD) | 1.90 (0.21) | 2.25 (0.22) | $t$=−9.00 | <0.001* |

$T$=Wilcoxon signed-ranks test; $t$=Paired $t$-test; *Statistically significant. SD: Standard deviation

**Table 2: Prolonged QTc interval and hypocalcemia within each clinical state (N=76)**

| Variable | Variable category | Clinical state | | Total, N (%) | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|
| | | VOC, *n* (%) | Steady state, *n* (%) | | | |
| Total calcium | Hypocalcemia | 34 (89.5) | 8 (21.1) | 42 (55.3) | 35.978 | <0.001* |
| | Normal calcium | 4 (10.5) | 30 (78.9) | 34 (44.7) | | |
| QTc interval | Prolonged | 26 (68.4) | 8 (21.1) | 34 (44.7) | 17.244 | <0.001* |
| | Normal | 12 (31.6) | 30 (78.9) | 42 (55.3) | | |

*=statistically significant; $\chi 2$= Chi square

**Table 3: Binary logistics of total calcium and QTc interval in the prediction of vaso-occlusive painful crises**

| Variable | Reference category | Wald test | OR (95% CI) | *P*-value |
|---|---|---|---|---|
| Total calcium | Hypocalcemia | 18.877 | 21.277 (0.012-0.187) | <0.001* |
| QTc interval | Prolonged QTc interval | 1.970 | 2.584 (0.103-1.457) | 0.160 |

*Statistically significant. CI: Confidence interval, OR: Odds ratio

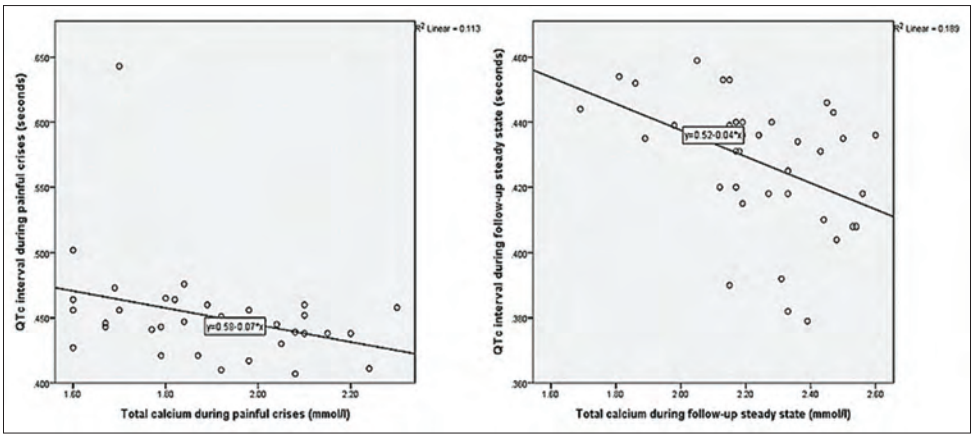


**Figure 1:** Correlations between Total calcium and corrected QT interval during VOC and follow-up steady state

may be due to possible increased calcium influx into the intracellular space as a result of reduction in plasma pH that occurs during VOC.[15] Intracellular accumulation of calcium was demonstrated by Etzion *et al*.[15] to be marked in the deoxygenated state like it occurs during VOC. Another factor that can explain hypocalcemia, especially during VOC, is low Vitamin D serum levels.[9] Diet poor in the precursors of Vitamin D and lack of exposure to sunlight lead to Vitamin D deficiency and consequent hypocalcemia. This is because Vitamin D stimulates active intestinal calcium absorption, which accounts for majority of its absorption, and calcium renal resorption.[22]

Hypocalcemia was excellently predicted by QTc interval which significantly but inversely correlated with total calcium during both the clinical states. These findings may be because hypocalcemia increases QTc interval above its threshold of 0.440 s.[11] Prolonged QTc interval may be as a result of enhanced late sodium current in the myocytes leading to increased intracellular accumulation of calcium, stabilization of the cell membrane, and subsequent delay in action potential propagation.[16] This prolonged QTc interval can lead to refractoriness in a part of the myocardium forming a nidus around which further waves of excitation propagate.[23] This can lead to ventricular arrhythmias and mortality.[14,24] The implication being that hypocalcemia may directly be associated with death.

However, there were subjects with hypocalcemia and normal QTc interval in this study. This may be explained by the different activities of the calcium pumps in the cell membranes of some SCA children.[15] Consequently, majority of the cells including myocytes may not be accumulated by calcium, hence normal QTc interval. Therefore, to a large extent, prolonged QTc substantiated the presence of hypocalcemia in this research though it failed to significantly predict VOC.

Hypocalcemia was observed more during VOC (89.5%) than in the follow-up steady state (21.1%) and hypocalcemia is 21 times more likely to occur during VOC than in steady state because it is significantly associated with VOC. Similarly, total calcium excellently predicted (AUC: 0.8–0.9) VOC.[25] This makes hypocalcemia a significant predictor of VOC. This finding is similar to the work by Nduka *et al*.[26] who showed that hypocalcemia occurs more during VOC than in steady state though their finding was not statistically significant.

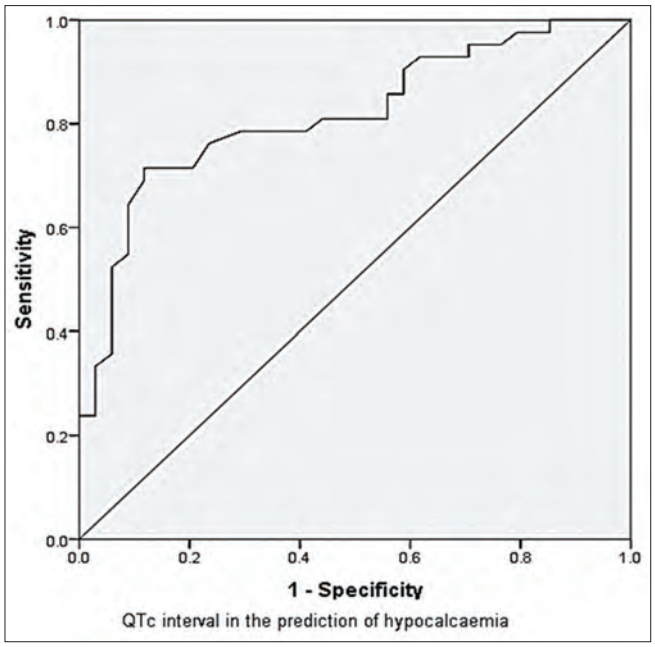


**Figure 2:** Receiver operating characteristics curve for corrected QT interval in the prediction of hypocalcaemia

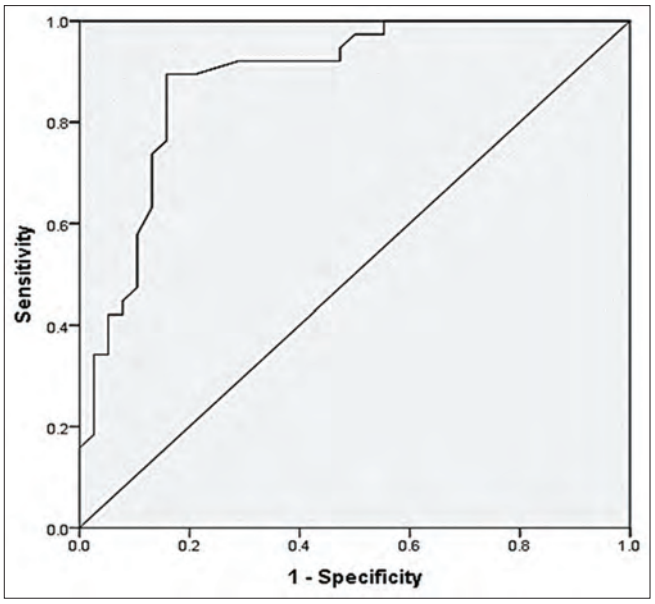


**Figure 3:** Receiver operating characteristic curve for total calcium in the prediction of the clinical state of VOC

Values of total calcium and albumin-adjusted calcium were very similar, so also values obtained in their comparisons with other variables. Hence, measuring only total calcium in SCA children suffices. Measurement of the active form of calcium (ionized calcium) could have been more relevant; however, this investigation is largely lacking in resource-poor settings. Thus, with accuracy levels of 89.5% sensitivity and 81.6% specificity, total calcium <2.13 mmol/l during the steady state may imply likelihood of VOC occurring.

## Conclusion

Hypocalcemia occurs in children with sickle cell anaemia especially during VOC. Despite the fact that no hypocalcaemic clinical feature was observed, low serum calcium is associated with prolonged QTc interval which can cause ventricular arrhythmia. It is pertinent to regularly assess total calcium in these children in other to prevent mortality. Therefore, introduction of routine ingestion of calcium, and Vitamin D to help in calcium intestinal absorption, may help to reduce the frequency of VOC and QTc prolongation.

### What is already known

Hypocalcemia occurs in sickle cell anemia. Hypocalcemia is associated with a prolonged QTc interval.

### What is unknown

Whether hypocalcemia substantially occurs during VOC.

### What the study adds

Hypocalcemia significantly occurs during VOC. It is 21 times more likely to occur during VOC than in steady state. Hypocalcaemia is also highly predictive of the clinical state of VOC. Calcium negatively correlates with QTc interval during VOC and steady state hence hypocalcaemia may predispose the SCA child to ventricular arrhythmias which are sequela of prolonged QTc interval.

### Acknowledgments

We are very grateful to Prof Friday Odey and the parents of these children for allowing their wards to take part in this study.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Steinberg MH. Sickle cell anemia, the first molecular disease: Overview of molecular etiology, pathophysiology and therapeutic approaches. Sci World J 2008;8:1295-324.
2. Ambe JP, Mava Y, Chama R, Farouq G, Machoko Y. Clinical features of sickle cell anaemia in Northern Nigerian children. West Afr J Med 2012;31:81-5.
3. Joiner CH. Cation transport and volume regulation in sickle red blood cells. Am J Physiol 1993;264:C251-70.
4. Litosch I, Lee KS. Sickle red cell calcium metabolism: Studies on $Ca^{2+}$ – $Mg^{2+}$ ATPase and Ca-binding properties of sickle red cell membranes. Am J Hematol 1980;8:377-87.
5. Luthra MG, Sears DA. Increased Ca++, Mg++ and Na++ K+ ATpase activities in erythrocytes of sickle cell anaemia. Blood 1982;60:1332-7.
6. Hänggi P, Makhro A, Gassmann M, Schmugge M, Goede JS, Speer O, *et al.* Red blood cells of sickle cell disease patients exhibit abnormally high abundance of N-methyl D-aspartate receptors mediating excessive calcium uptake. Br J Haematol 2014;167:252-64.
7. Rhoda MD, Apovo M, Beuzard Y, Giraud F. $Ca^{2+}$permeability in deoxygenated sickle cells. Blood 1990;75:2453-8.
8. Oladipo OO, Temiye EO, Ezeaka VC, Obomanu P. Serum magnesium, phosphate and calcium in nigerian children with sickle cell disease. West Afr J Med 2005;24:120-3.
9. Lee MT, Licursi M, McMahon DJ. Vitamin D defciency and acute vaso-occlusive complications in children with sickle cell disease. Pediatr Blood Cancer 2015;62:643-7.
10. Ohene-Frempong K, Stallings VA, Kawchak DA, Schall JI, Zemel BS.

Adequacy of dietary intake declines with age in children with sickle cell disease. J Am Diet Assoc 2007;107:843-8.
11. Park MK. Electrocardiography. In: Park' s Pediatric Cardiology for Practitioners. 6th ed. Philadelphia: Elsevier Saunders; 2014. p. 40-65.
12. Bode-Thomas F, Hyacinth HI, Ogunkunle O, Omotoso AB. The QT interval in Nigerian children with sickle cell anaemia. Trop Cardiol 2003;113:9-12.
13. Nlemadim AC, Okpara HC, Anah MU, Odey FA, Meremikwu MM. Myocardial injury in patients with sickle cell anaemia and myocardial ischaemia in Calabar, Nigeria. Paediatr Int Child Health 2020;40:231-7.
14. Indik JH, Nair V, Rafikov R, Nyotowidjojo IS, Bisla J, Kansal M, *et al.* Associations of prolonged QTc in sickle cell disease. PLoS One 2016;11:e0164526.
15. Etzion Z, Tiffert T, Bookchin RM, Lew VL. Effects of deoxygenation on active and passive $Ca^{2+}$ transport and on the cytoplasmic $Ca^{2+}$ levels of sickle cell anemia red cells. J Clin Invest 1993;92:2489-98.
16. Yu S, Li G, Huang CL, Lei M, Wu L. Late sodium current associated cardiac electrophysiological and mechanical dysfunction. Pflugers Arch 2018;470:461-9.
17. Ballas SK, Lieff S, Benjamin LJ, Dampier CD, Heeney MM, Hoppe C, *et al*. Critical review: Definitions of the phenotypic manifestations of sickle cell disease. Am J Hematol 2010;85:6-13.
18. Ballas SK. More definitions in sickle cell disease: Steady state v base line data. Am J Hematol 2012;87:338-9.
19. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, *et al.* Fourth universal definition of myocardial infarction. J Am Coll Cardiol 2018;72:2231-64.
20. Payne RB, Carver ME, Morgan DB. Interpretation of serum total calcium: Effects of adjustment for albumin concentration on frequency of abnormal values and on detection of change in the individual. J Clin Pathol 1979;32:56-60.
21. Roberts WL, McMillinM GA, Burtis CA, Bruns DE. Reference information for the clinical laboratory. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. Missouri: Elsevier; 2012. p. 2131-88.
22. Barrett KE, Barman SM, Brooks HL, Yuan J. Hormonal control of calcium & phosphate metabolism & the physiology of bone. In: Ganong's Review of Medical Physiology. 26th ed. New York: McGraw-Hill Education; 2019. p. 369-70.
23. Park MK. Rhythms originating in the ventricle. In: Park' s Pediatric Cardiology for Practitioners. 6th ed. Philadelphia: Elsevier Saunders; 2014. p. 430-3.
24. Upadhya B, Ntim W, Brandon Stacey R, Henderson R, Leedy D, O'Brien FX, *et al.* Prolongation of QTc intervals and risk of death among patients with sickle cell disease. Eur J Haematol 2013;91:8-11.
25. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol 2010;5:1315-6.
26. Nduka N, Kazem Y, Saleh B. Variation in serum electrolytes and enzyme concentrations in patients with sickle cell disease. J Clin Pathol 1995;48:648-51.