The Inflammatory Response to Surgery in Sickle Cell Disease Patients Undergoing Cholecystectomy

Adewale O. Adisa, FWACS, FMCS, Tewogbade A. Adedeji, FMCPath, FWACP, Rahman A. Bolarinwa, MBChB, FMCPath, Temilola O. Owojuyigbe, MBBS, FMCPath, Olusola A. Jeje, MBChB, FMCPath, James Glasbey, MBBCh, MRCS, Norah O. Akinola, PhD, FMCPath

ABSTRACT

Background and Objectives: Patients with sickle cell anemia (SCA) may have elevated inflammatory markers in health, and this may be heightened after open operations. The inflammatory response of patients with SCA after minimally invasive surgeries has not been fully explored.

Patients and Methods: Consecutive patients with SCA and with hemoglobin AA (HbAA) undergoing laparoscopic cholecystectomy for acute cholecystitis were recruited into the study. Blood samples were taken before induction of anesthesia (0-h); at 4, 12, 24, and 48 h; and on postoperative day 7. Samples were analyzed for serum C-reactive protein and interleukin (IL)-1 through IL-18.

Results: Twenty-three patients, including 9 with SCA and 14 with HbAA, were recruited with 4 cases performed by open laparotomy. At 0-h, proinflammatory IL-1 levels (6.1 versus 4.8) and C-reactive protein levels (32.5 versus 26.6) were higher in patients with hemoglobin SS (HbSS) than in patients with HbAA, respectively. Over time, inflammatory markers were generally higher at each time-point for patients with HbSS compared with patients with HbAA for both proinflammatory and anti-inflammatory cytokines, rising immediately after surgery and up to 48 hours, then returning to baseline by postoperative day 7. There was a higher mean IL-1 level across all time-points in the HbSS group than in the HbAA group ($P = .04$).

Conclusion: This exploratory study found an enhanced inflammatory response to cholecystectomy in patients with SCA compared with patients with HbAA. Minimally invasive surgical strategies for this patient group may help to mediate this response.

Key Words: Sickle cell disease, acute phase reaction, gallbladder disease, minimally invasive surgery, laparoscopic surgery.

INTRODUCTION

Sickle cell anemia (SCA) is associated with a high incidence of gallstone disease and its sequelae, and many patients with SCA eventually therefore require cholecystectomy.1 In the past, open cholecystectomy in patients with SCA was associated with high morbidity and mortality.2 The advent of minimally invasive techniques for the treatment of cholelithiasis in patients with SCA has led to a significant reduction in morbidity after cholecystectomy around the world. For example, a retrospective study of 427 adults with SCA undergoing elective laparoscopic cholecystectomy (LC) over a 13-y period in Saudi Arabia reported a 7% complication rate and no deaths.3 However, access to training in laparoscopic surgery, equipment, and maintenance in low- and middle-income countries is limited, with open cholecystectomy remaining the standard of practice in many countries.

Patients with SCA have been previously demonstrated to have elevated inflammatory markers even in health, indicating dysregulated inflammation.4–6 In the setting of acute cholecystitis, a proinflammatory cytokine cascade may be seen of magnitude
beyond that of wild-type (hemoglobin AA [HbAA]) patients. The influence of this heightened inflammatory response on surgical outcomes in patients with SCA with acute cholecystitis has not been fully explored. Studies have profiled changes in cytokine of wild-type patients after laparoscopy, but this has not been well studied in patients with SCA (hemoglobin SS [HbSS]). Laparoscopy may provide additional benefit over open cholecystectomy in patients with SCA by reducing systemic inflammation and mitigating against the enhanced surgical stress response.

The primary aim of this study was to assess differences in levels of proinflammatory and anti-inflammatory acute phase proteins after cholecystectomy in patients with HbAA and patients with HbSS. The secondary aims were to explore relationships between acute phase protein responses, and differences in the cytokine response in patients who received open cholecystectomy compared with patients who received laparoscopic cholecystectomy.

METHODS
A prospective cohort study of adult (>16 y) patients with HbAA and patients with HbSS undergoing open or laparoscopic cholecystectomy for AC were recruited over a 1-year period (October 1, 2015, to September 31, 2016) in a single general surgery unit of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. This is a large tertiary hospital in an urban setting with a referral service for gallbladder surgery. Informed consent for involvement in the study was provided in line with International Committee on Harmonisation Good Clinical Practice guidelines, and written consent forms were completed by all willing participants. The study was approved by the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex with protocol number ERC/201/08/06. Hemoglobin electrophoresis was used to confirm hemoglobin phenotype for both sickle and non-sickle cell status.

Patients with ultrasonographically proven cholelithiasis with features of acute cholecystitis were eligible. Those with radiological features of chronic calculous cholecystitis and those with choledocholithiasis were excluded from the study. Patients were selected for open or laparoscopic cholecystectomy based on disease severity; open surgery was chosen when there was radiological evidence of markedly contracted gallbladder, extensive pericholecystic fluid on ultrasound, or in instances of gangrenous gallbladder.

Laparoscopic cholecystectomy was performed by a single surgeon (the first author) using a conventional 4-port tech-

---

Figure 1. Cohort recruitment and operation types. SCA = sickle cell anemia (HbSS). HbAA = wild-type hemoglobin.
nique under carbon dioxide pneumoperitoneum. General anesthesia was employed in all cases with intraoperative noninvasive hemodynamic monitoring. Postoperative hydration, antibiotics, and oxygen therapy were continued in all patients with adequate opioid analgesia for pain control. All patients were treated with intravenous antibiotics for a period of 24 h according to local formulary guidelines.

**Laboratory Methods, Accuracy, and Precision Controls**

Study patients provided 5-mL samples of venous blood at 6 time-points: before induction of anesthesia (0-h); at 4, 12, 24, and 48 h; and on postoperative day 7. Samples were analyzed for serum C-reactive protein (CRP) and interleukin (IL)-1 through IL-18, using standardized assays. All blood samples taken from participants were centrifuged (3500 g for 5 min). The supernatant serum was separated and frozen at –80°C until assayed, within the time limit of analyte stability as specified in the assay standard operating procedures. IL-6 was assayed by using high performance liquid chromatography (HPLC) (Waters 616/626 HPLC; Waters Inc., Milford, MA).

High-sensitivity (hs)CRP enzyme-linked immunosorbent assay (ELISA) kits and control sera were purchased from Monobind Inc. (Lake Forest, CA). Quantitative ELISAs based on competitive immunoassay principles were used. Analytical accuracy and precision were ensured by simultaneous analyses of commercially prepared control sera (at levels in the low, normal, and elevated ranges) for monitoring assay performance in each batch of samples that was analyzed. All serum specimens were analyzed in batches. Intra-assay, interassay, and day-to-day coefficients of variation were estimated for each batch of the hsCRP and were within allowable limits of acceptance for the analyte.

**Statistical Analysis**

Data were collected on patient demographics, sickle cell status (HbAA, HbSS), operative approach (open, laparoscopic), and inflammatory mediator levels over time. ILs and CRP were grouped into proinflammatory (IL-1, IL-6, IL-8, IL-12, CRP) and anti-inflammatory (IL-4, IL-10, IL-1, total IL) mediators based on published literature. Testing of normality for continuous variables was performed using Shapiro-Wilk tests. Differences between main explanatory variables (HbAA versus HbSS, open versus laparoscopic cholecystectomy) were described by using percentages, mean (normal) and standard deviation, or median-averages and interquartile ranges (nonparametric). Testing for significance between groups was performed using unpaired Student t tests for parametric data, Wilcoxon rank sum tests for nonparametric data fields, and χ² tests for categorical data. An α level (risk of type I error) of <.05 was accepted as significant.

**RESULTS**

During the study period, 23 eligible patients underwent cholecystectomy for acute cholecystitis and were con-
sented for study inclusion. The mean age was 41 y (range 20 to 69 y), and 20 (87.0%) female patients and 3 (13.0%) male patients were included. Nineteen patients underwent laparoscopic cholecystectomy, and 4 underwent open cholecystectomy. Nine patients had HbSS and 14 had HbAA (Figure 1).

Differences between baseline demographics and operative approach for patients with HbSS and patients with HbAA are shown in Table 1. The patients with HbSS were more likely to be younger ($P < .001$) and male ($P < .001$) compared with the patients with HbAA, but both groups had a representative proportion of laparoscopic (7 versus 12, respectively) and open operations (2 versus 2, respectively; $P = .16$).

**Interaction Between Acute Phase Proteins Around the Time of Surgery**

The interaction between measured acute phase protein levels is shown in Figure 2. Strong correlations were seen...
between IL-6 and IL-8 (<i>R</i>2 = 0.91), IL-6 and IL-10 (<i>R</i>2 = 0.71), IL-8 and IL-10 (<i>R</i>2 = 0.90), and IL-11 and IL-12 (<i>R</i>2 = 0.79) levels.

Differences Between Inflammatory Responses of Patients With SCA and Patients With Wild-Type

There was a higher mean IL-1 level across all time-points in the HbSS group than in the HbAA group (<i>P</i> = .04, Table 2). No other significant differences were observed. The association between mean acute phase protein levels and sickle cell status is displayed in cubic spline curves in Figure 3.

Acute phase protein levels and their variation over time overall and split by HbAA and HbSS groups are displayed in Table 3. Across all included patients, only IL-1 and CRP levels were found to respond significantly over time after surgical stress (<i>P</i> < .001). At 0-h (induction of anesthesia), proinflammatory IL-1 levels (6.1 versus 4.8 pg/mL) and CRP levels (32.5 μg/mL versus 26.6 μg/mL) were higher in patients with HbSS than in patients with HbAA, respectively, but this did not reach statistical significance. In comparing trends over time between wild-type (HbAA) and sickle cell (HbSS) patients, inflammatory markers were generally higher at each time point for patients with HbSS compared with patients with HbAA for both proinflammatory and anti-inflammatory cytokines, rising immediately after surgery and up to 48 h, then returning to baseline by postoperative day 7. The difference seen in overall IL-1 levels between patients with HbAA and patients with HbSS was not significant at any individual time-point, and there were no other significant differences in inflammatory mediator levels at selected time-points (Figure 4).

**Table 2.** Overall Acute Phase Protein Levels in Postoperative HbAA and HbSS Patients

| Sickle Cell Status | <i>H</i>bAA | <i>H</i>bSS | <i>P</i> value |
|-------------------|------------|------------|-------------|
| **<i>H</i>bAA**    |            |            |             |
| Pro-inflammatory  |            |            |             |
| IL-1              | 6.1 (2.8)  | 7.2 (2.5)  | .041*       |
| IL-6              | 0.1 (0.1)  | 0.1 (0.1)  | .498        |
| IL-8              | 0.8 (0.8)  | 0.8 (0.8)  | .438        |
| IL-12             | 0.7 (0.7)  | 0.7 (0.7)  | .322        |
| CRP               | 65.8 (61.2)| 62 (58.5)  | .869        |
| **Anti-inflammatory** |            |            |             |
| IL-4              | 0.8 (0.4)  | 0.8 (0.5)  | .393        |
| IL-10             | 0.3 (0.7)  | 0.3 (0.7)  | .998        |
| IL-11             | 0.2 (0.1)  | 0.2 (0.2)  | .784        |

Hb = hemoglobin; HbAA = wild-type; HbSS = sickle cell anemia; IL = interleukin; CRP = C-reactive protein; POD = postoperative day.

Averages presented as mean (standard deviation).

<i>P</i> values derived from Student <i>t</i> test for normal with <i>α</i> < .05 accepted as significant.

Differences Between Inflammatory Responses in Laparoscopic and Open Cholecystectomy

The mean values of the acute phase proteins in patients undergoing laparoscopic and open cholecystectomy are shown in Table 4. Significant differences in the mean or median average levels of IL-1 (6.5 versus 7.4 pg/mL, respectively, <i>P</i> = .046) and IL-12 (0.8 versus 0.5 pg/mL, respectively, <i>P</i> = .027) were seen between the operative approach groups. There were no other statistically significant differences in the median averages of other ILs or CRP.
DISCUSSION

Summary of Key Findings

This study demonstrates an enhanced IL-1–mediated inflammatory response to surgery in patients with SCA undergoing open and laparoscopic cholecystectomy versus wild-type patients. Although this was not replicated at individual time-points, these analyses are likely underpowered within the available sample size. Patients undergoing a laparoscopic approach had lower magnitude response to the surgical insult; minimally invasive approaches may present a method to mitigate against heightened inflammatory responses to surgery in sickle cell patients. Our data also highlight IL-1, IL-12, and CRP as important markers for future investigation.

Table 3.
Variation in Proinflammatory and Anti-Inflammatory Protein Levels Over Time in Sickle Cell Disease and Wild-type Patients Undergoing Cholecystectomy

| Protein | Sickle Cell Status | 0 h | 4–24 h | 24–48 h | POD7 | P value |
|---------|-------------------|-----|--------|---------|------|---------|
|         |                   |     |        |         |      |         |
| Pro-inflammatory mediators | | | | | | |
| IL-1    | Overall           | 5.3 (3.5) | 6 (2.3) | 7.1 (2.5) | 8.2 (2.2) | <.001* |
|         | Wild-type (HbAA)  | 4.8 (3.1) | 5.6 (2.5) | 6.8 (2.7) | 7.4 (2.4) | .037*  |
|         | Sickle cell (HbSS)| 6.1 (4) | 6.6 (1.7) | 7.7 (2.2) | 9.4 (1.5) | .001*  |
| IL-6    | Overall           | 0.1 (0) | 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | .766   |
|         | Wild-type (HbAA)  | 0.1 (0) | 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | .898   |
|         | Sickle cell (HbSS)| 0.1 (0.1)| 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | .919   |
| IL-8    | Overall           | 0.7 (0.6) | 0.8 (0.8) | 0.8 (0.8) | 1 (1.2) | .975   |
|         | Wild-type (HbAA)  | 0.7 (0.5) | 0.8 (0.8) | 0.9 (0.8) | 0.9 (1.2) | .953   |
|         | Sickle cell (HbSS)| 0.8 (0.8) | 0.8 (0.7) | 0.7 (0.8) | 1.1 (1.4) | .934   |
| IL-12   | Overall           | 0.6 (0.4) | 0.8 (0.7) | 0.7 (0.6) | 0.7 (0.3) | .986   |
|         | Wild-type (HbAA)  | 0.5 (0.2) | 0.8 (0.6) | 0.8 (0.7) | 0.7 (0.2) | .524   |
|         | Sickle cell (HbSS)| 0.9 (0.6) | 0.8 (0.9) | 0.6 (0.5) | 0.6 (0.5) | .656   |
| CRP     | Overall           | 29 (36.1) | 54.4 (54.7) | 95.6 (60.4) | 54.7 (65.2) | <.001* |
|         | Wild-type (HbAA)  | 26.6 (38.2) | 67.4 (63.1) | 92.9 (61.9) | 34.4 (38.6) | .002*  |
|         | Sickle cell (HbSS)| 32.5 (34.9) | 34.8 (31.7) | 99.8 (59.8) | 83.2 (87.9) | .004*  |
| Anti-inflammatory mediators | | | | | | |
| IL-4    | Overall           | 0.8 (0.4) | 0.8 (0.6) | 0.8 (0.4) | 0.8 (0.4) | .851   |
|         | Wild-type (HbAA)  | 0.7 (0.3) | 0.8 (0.5) | 0.8 (0.4) | 0.8 (0.2) | .89    |
|         | Sickle cell (HbSS)| 0.9 (0.5) | 0.9 (0.6) | 0.8 (0.4) | 0.9 (0.6) | .93    |
| IL-10   | Overall           | 0.3 (0.7) | 0.3 (0.6) | 0.3 (0.6) | 0.5 (1.2) | .935   |
|         | Wild-type (HbAA)  | 0.2 (0.5) | 0.3 (0.7) | 0.3 (0.6) | 0.6 (1.5) | .523   |
|         | Sickle cell (HbSS)| 0.4 (0.9) | 0.3 (0.6) | 0.3 (0.7) | 0.5 (1) | .727   |
| IL-11   | Overall           | 0.2 (0.1) | 0.2 (0.2) | 0.2 (0.2) | 0.2 (0.1) | .87    |
|         | Wild-type (HbAA)  | 0.1 (0.1) | 0.2 (0.2) | 0.2 (0.2) | 0.2 (0.1) | .325   |
|         | Sickle cell (HbSS)| 0.2 (0.1) | 0.2 (0.2) | 0.2 (0.1) | 0.2 (0.1) | .688   |

Hb = hemoglobin; HbAA = wild-type; HbSS = sickle cell anemia; IL = interleukin; CRP = C-reactive protein; POD = postoperative day.
Averages presented as mean (standard deviation).
*P values derived from Student t test for normal with α < .05 accepted as significant.
ure studies examining the inflammatory response to surgery.

**Comparison With Previous Literature**

The finding of proinflammatory cytokines, particularly IL-1 and CRP, being higher at baseline (0-h) and at each time-point in SCA compared with the wild-type patients with HbAA is consistent with previous reports showing significant elevations of various proinflammatory cytokines among patients with SCA in their steady state. Most of these studies also report no significant difference in the levels of the anti-inflammatory cytokines among these patients, similar to our findings. Other studies have shown that the altered cytokine levels correlate with altered hematological and clinical parameters. This implies that even in steady state, patients with SCA have chronic inflammation, which affects their clinical and physiological state, requiring perioperative considerations. A previous study of outcomes of laparoscopic cholecystectomy in sickle cell patients highlighted preoperative blood transfusion as a factor that may reduce intraoperative and postoperative complications. Subsequent studies on the subject has been inconclusive due to the low quality of the evidence. The possible immune depression induced by such transfusion and its implications on the clinical outcome of patients with SCA should be explored in future studies.

There is paucity of data comparing immunological responses of patients with SCA in the open versus the laparoscopic approach, with some reports highlighting only shorter hospitalization but similar overall complications between the 2. In patients with HbAA, however, previous studies comparing open and laparoscopic cholecystectomies have highlighted immunological advantages of the laparoscopic approach. We found a similar trend in the current study with significant elevation of IL-1 in patients having the open compared with the laparoscopic approach, although the proportion undergoing open operation was very low due to the low indications for open cholecystectomies in both the patients with SCA and the patients with HbAA. Overall, we identified IL-1, IL-12, and CRP as important markers for future studies examining the inflammatory response to surgery.

**Table 4.**

| Operative Approach | Laparoscopic | Open | P value |
|--------------------|--------------|------|---------|
| **Pro-inflammatory** | | | |
| IL-1 | 6.3 (2.9) | 7.4 (1.7) | .046* |
| IL-6 | 0.1 (0.1) | 0.1 (0.1) | .998 |
| IL-8 | 0.8 (0.7) | 1.1 (1.1) | .635 |
| IL-12 | 0.8 (0.6) | 0.5 (0.5) | .027* |
| CRP | 61.3 (58.1) | 75.6 (66.8) | .203 |
| **Anti-inflammatory** | | | |
| IL-4 | 0.8 (0.4) | 0.8 (0.5) | .815 |
| IL-10 | 0.2 (0.6) | 0.6 (1) | .663 |
| IL-11 | 0.2 (0.2) | 0.2 (0.1) | .848 |

Hb = hemoglobin; HbAA = wild-type; HbSS = sickle cell anemia; IL = interleukin; CRP = C-reactive protein; POD = postoperative day.

Averages presented as mean (standard deviation).

*P values derived from Student t test for normal with α < .05 accepted as significant.
**Strengths and Limitations**

This study provides valuable data to support the use of minimally invasive cholecystectomy in the context of SCA. Serum analysis was performed with a high level of quality assurance and at multiple time-points in the postoperative period, enabling high-fidelity temporal analysis. The data also highlight useful targets for future research in perioperative inflammation by characterizing relationships between inflammatory mediator levels in the postoperative period. Future studies targeting different operative techniques and procedures in patients with hemoglobinopathies can build on the specific cytokine variations demonstrated in this study. The study has some important limitations. First, no clinical data were collected, so correlation of inflammation levels to patient-level outcomes was not possible. Second, selection bias existed in selection of patients for laparoscopic surgery; the balance of operative approach in the HbAA and SCA groups, however, was good, so this is unlikely to have affected the principal findings. Finally, the small sample size meant that analyses were underpowered to detect differences in acute phase protein levels at individual time-points or within subgroups. The data, therefore, should be considered as hypothesis generating when targeting future research.

**CONCLUSION**

This study demonstrates an enhanced inflammatory response to cholecystectomy in SCA compared with wild-type. Minimally invasive surgical strategies for this patient group may help to mediate this and mitigate against adverse outcomes.

**References:**

1. Séguier-Lipszyc E, de Llagausie P, Benkerrou M, Di Napoli S, Aigrain Y. Elective laparoscopic cholecystectomy. Treatment of choice for cholelithiasis in children with sickle cell disease? *Surg Endosc.* 2001;15:501–304.

2. Leandros E, Kymionis GD, Konstadoulakis MM, et al. Laparoscopic or open cholecystectomy in patients with sickle cell disease: Which approach is superior? *Eur J Surg.* 2000;166:859–61.

3. Al-Mulhim AS, Al-Mulhim AA. Laparoscopic cholecystectomy in 427 adults with sickle cell disease. *Eur J Surg.* 2009;2:1599–1602.

4. Bourantas KL, Dalekos GN, Makis A, Chaidos A, Tsilis S, Mavridis A. Acute phase proteins and interleukins in steady state sickle cell disease. *Eur J Haematol.* 1998;61:49–54.

5. Akinola NO, Stevens SM, Franklin IM, Nash GB, Stuart J. Subclinical ischaemic episodes during the steady state of sickle cell anaemia. *J Clin Pathol.* 1992;45:902–906.

6. Makis AC, Hatzimichael EC, Mavridis A, Bourantas KL. Alpha-2-macroglobulin and interleukin-6 levels in steady-state sickle cell disease patients. *Acta Haematol.* 2000;104:164–168.

7. Cavalcante JE, Machado RP, Laurentino MR, et al. Clinical events and their relation to the tumor necrosis factor-alpha and interleukin-10 genotypes in sickle-cell anemia patients. *Hematol Oncol Stem Cell Ther.* 2016;9:14–19.

8. Cajado C, Cerqueira BA, Couto FD, et al. TNF-alpha and IL-8: Serum levels and gene polymorphisms are associated with classical biomarkers and medical history in children with sickle cell anemia. *Cytokine.* 2011;56:312–317.

9. Grande M, Tucci GF, Adorisio O, et al. Systemic acute-phase response after laparoscopic and open cholecystectomy. *Surg Endosc.* 2002;16:313–316.

10. Krog AH, Sahba M, Pettersen EM, et al. Comparison of the acute-phase response after laparoscopic versus open aortobifemoral bypass surgery: A substudy of a randomized controlled trial. *Vasc Health Risk Manage.* 2016;12:371–378.

11. Tsamis D, Theodoropoulos G, Stamopoulos P, et al. Systemic inflammatory response after laparoscopic and conventional colectomy for cancer: A matched case-control study. *Surg Endosc.* 2012;26:1436–1443.

12. Akdis M, Burgler S, Crameri R, et al. Interleukins, from 1 to 37, and interferon-y: receptors, functions, and roles in diseases. *J Allergy Clin Immunol.* 2011;127:701–21.e1–70.

13. Lanaro C, Franco-Penteado CF, Albuquerque DM, Saad ST, Conran N, Costa FF. Altered levels of cytokines and inflammatory mediators in plasma and leukocytes of sickle cell anemia patients and effects of hydroxyurea therapy. *J Leukoc Biol.* 2009;85:235–242.

14. Bandeira IC, Rocha LB, Barbosa MC, et al. Chronic inflammatory state in sickle cell anemia patients is associated with HBB* S haplotype. *Cytokine.* 2014;65:217–221.

15. Alagbe AE, Olaniyi JA, Aworanti OW. Adult sickle cell anemia patients in bone pain crisis have elevated pro-inflammatory cytokines. *Mediterr J Hematol Infect Dis.* 2018;10:e2018017.

16. Garadah TS, Jaradat AA, AlAlawi ME, Hassan AB, Sequeira J. Severe acute-phase response after laparoscopic and open aortobi-femoral surgery: A substudy of a randomized controlled trial. *Vasc Health Risk Manage.* 2016;12:371–378.
18. Keikhaei B, Mohseni AR, Norouzirad R, et al. Altered levels of pro-inflammatory cytokines in sickle cell disease patients during vaso-occlusive crises and the steady state condition. *Eur Cytokine Netw.* 2013;24:45–52.

19. Akinlade KS, Atere AD, Rahamon SK, Olaniyi JA. Serum levels of copeptin, C-reactive protein and cortisol in different severity groups of sickle cell anaemia. *Niger J Physiol Sci.* 2013;28:159–164.

20. Aneke JC, Adegoke AO, Osho PO, et al. Blood pressure indices and disease severity in patients with sickle cell anaemia. *Niger J Med.* 2016;25:60–69.

21. Akinlade KS, Atere AD, Olaniyi JA, Rahamon SK, Adewale CO. Serum copeptin and cortisol do not accurately predict sickle cell anaemia vaso-occlusive crisis as C-reactive protein. *PLoS One.* 2013;8:e77913.

22. Haberkern CM, Neumayr LD, Orringer EP, et al. Cholecystectomy in sickle cell anemia patients: Perioperative outcome of 364 cases from the National Preoperative Transfusion Study Group. *Blood.* 1997;89:1533–1542.

23. Aziz AM, Meshikhes AW. Blood transfusion in patients with sickle cell disease requiring laparoscopic cholecystectomy. *JSLS.* 2011;15:480–485.

24. Estcourt IJ, Fortin PM, Trivella M, Hopewell S. Preoperative blood transfusions for sickle cell disease. *Cochrane Database Syst Rev.* 2016;4:CD003149.

25. Goers T, Panepinto J, DeBaun M, et al. Laparoscopic versus open abdominal surgery in children with sickle cell disease is associated with a shorter hospital stay. *Pediatr Blood Cancer.* 2008;50:603–606.

26. Sandoval C, Stringel G, Ozkaynak F, Tugal O, Jayabose S. Perioperative management in children with sickle cell disease undergoing laparoscopic surgery. *JSLS.* 2002;6:29–33.

27. Schietroma M, Carlei F, Franchi L, et al. A comparison of serum interleukin-6 concentrations in patients treated by cholecystectomy via laparotomy or laparoscopy. *Hepatogastroenterology.* 2004;51:1595–1599.

28. Sista F, Schietroma M, Santis GD, et al. Systemic inflammation and immune response after laparotomy vs laparoscopy in patients with acute cholecystitis, complicated by peritonitis. *World J Gastrointest Surg.* 2013;5:73–82.