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

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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.

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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.[7,8] 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]).[9–11] 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 calculus 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-
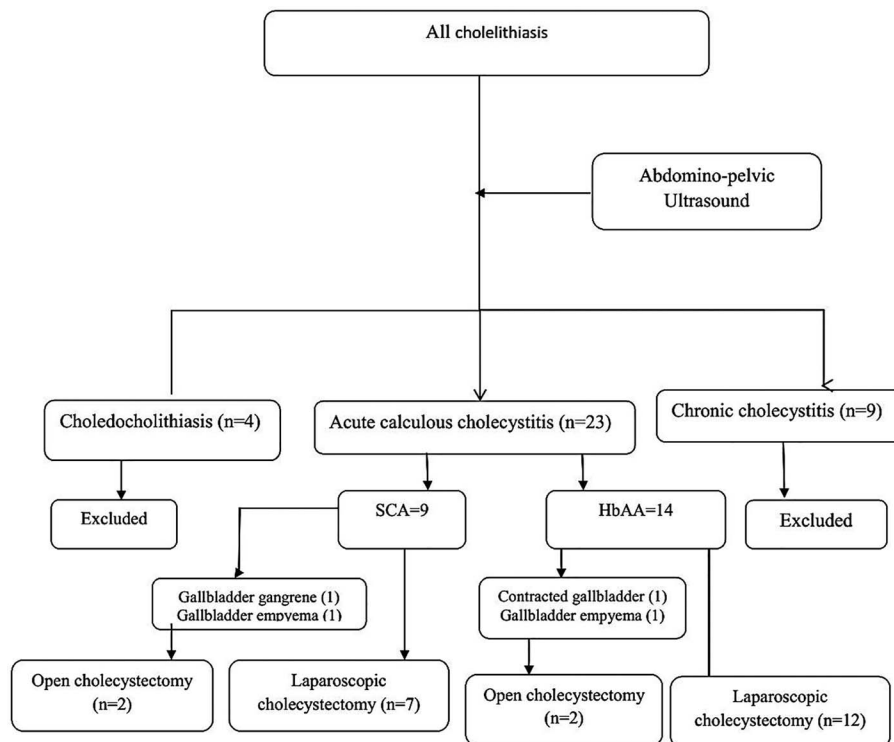

Figure 1. Cohort recruitment and operation types. SCA = sickle cell anemia (HbSS). HbAA = wild-type hemoglobin.

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 (3500g 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.[12] 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 per-

Table 1.
Patient- and Operation-Specific Factors of Patients Undergoing Cholecystectomy, Grouped by Sickle Cell Status

Factor	Total	Sickle Cell Status		P value
		HbAA	HbSS	
Gender				
Male	3	0	3	<.001*
Female	19	14	6	
Age group				
<30	4	0	4	<.001*
30–40	8	3	5	
41–55	6	6	0	
56–70	5	5	0	
Approach				
Laparoscopic	19	12	7	.157
Open	4	2	2	
Total		14	9	

HbAA = wild-type; HbSS = sickle cell anemia.
Age present in years of age.
An α level <.05 was accepted as significant.

formed using unpaired Student t tests for parametric data, Wilcoxon rank sum tests for nonparametric data fields, and χ^2 tests for categorical data. An α level (risk of type I error) of <.05 was accepted as significant.

Relationships between acute phase protein levels were displayed using scatterplots and histograms and correlation was quantified using Pearson's correlation coefficient. A strong correlation was defined as a correlation coefficient >0.5 (positive) or <−0.5 (negative). Cubic spline curves were plotted by using a locally estimated scatterplot smoothing function to demonstrate relationships between smoothed conditional mean levels of inflammatory mediators and a patient's sickle cell status or operative approach. To explore the relationships of inflammatory mediators over time and groups of interest, scatterplots were created of point estimates with 95% confidence intervals displayed as error bars. Statistical analyses were undertaken using R Project for Statistical Computing (R Foundation, Vienna University, Vienna, Austria) with the packages forcats, Hmisc, tidyverse, ggplot, gridExtra, tableone, scales, and dplyr.

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
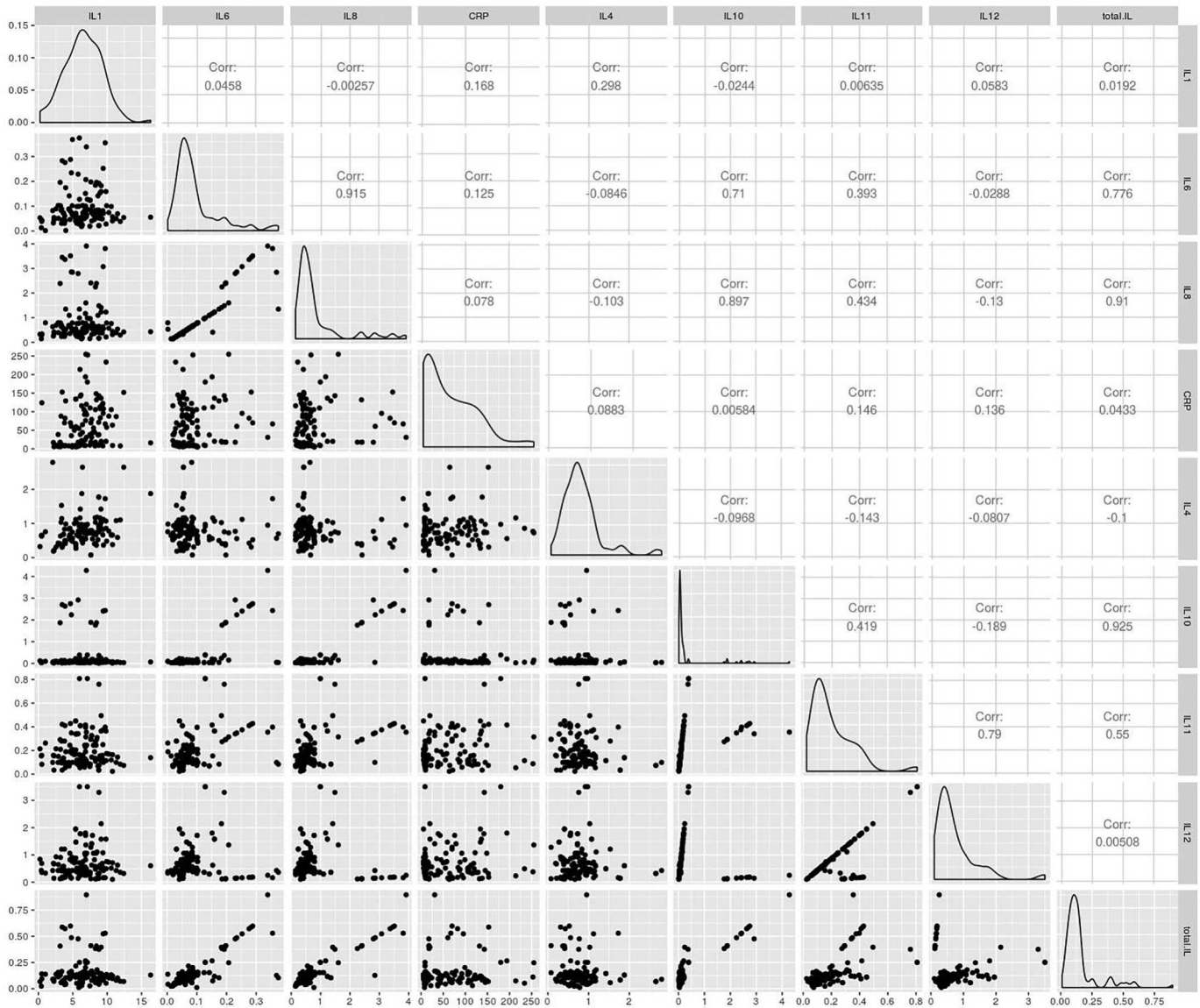

Figure 2. Correlation between overall acute phase protein levels. Corr = Pearson correlation coefficient (−1 to +1); >+0.5 and <−0.5 were accepted as strong correlation. Scatterplots demonstrate association between protein levels across all time-points. Histograms display spread and normality.

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

	Sickle Cell Status		*P* value
	HbAA	*HbSS*	
	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).

P values derived from Student *t* test for normal with $\alpha < .05$ accepted as significant.

Figure 3. Cubic spline curves demonstrating relationship between pro and anti-inflammatory acute phase proteins and sickle cell status. IL = interleukin. CRP = C-reactive protein. Data presented as smoothed conditional mean levels of included interleukins and CRP. Sickle cell anemia (HbSS) transformed into $y = 2$, and HbAA transformed into as $y = 1$.

between IL-6 and IL-8 (+0.91), IL-6 and IL-10 (+0.71), IL-8 and IL-10 (+0.90), and IL-11 and IL-12 (+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 ($P = .04$, **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 ($P < .001$). 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 proin-
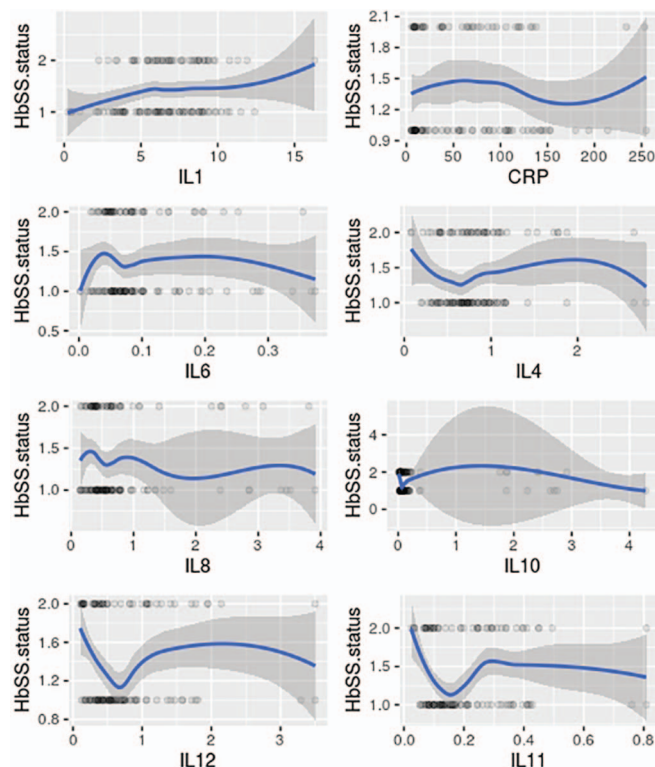
flammatory and anti-inflammatory cytokines, rising immediately after surgery and up to 48 h, then returning to baseline by postoperative day 7. The difference seen in overall IL-1 levels between patients with HbAA and patients with HbSS was not significant at any individual time-point, and there were no other significant differences in inflammatory mediator levels at selected time-points (**Figure 4**).

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 (6.3 versus 7.4 pg/mL, respectively, $P = .046$) and IL-12 (0.8 versus 0.5 pg/mL, respectively, $P = .027$) were seen between the operative approach groups. There were no other statistically significant differences in the median averages of other ILs or CRP.

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	Postoperative Serum Measurements				*P* value
		0 h	4–24 h	24–48 h	POD7	
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 $\alpha < .05$ accepted as significant.

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 fut-
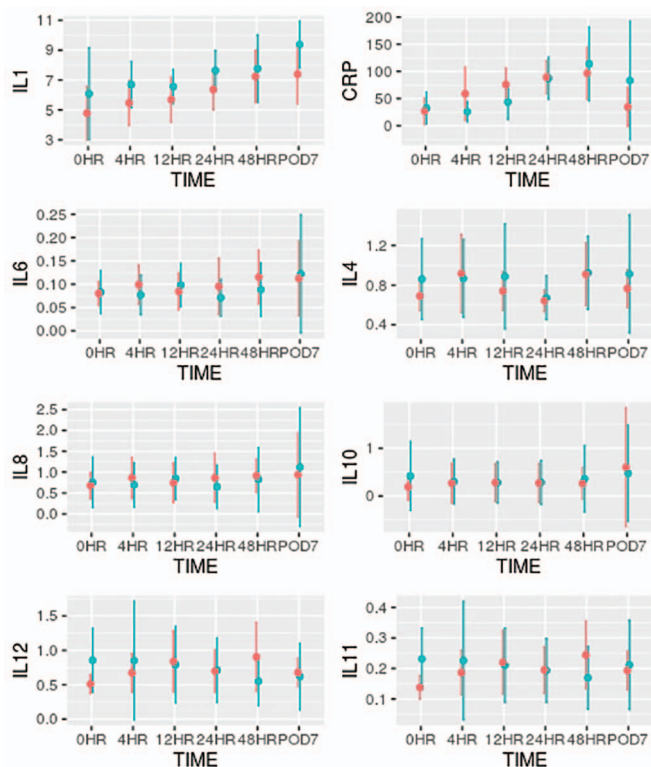
Figure 4. Variations in levels of proinflammatory and anti-inflammatory interleukins and C-reactive protein over time after cholecystectomy in sickle cell (blue) and HbAA (red) patients. IL = interleukin. CRP = C-reactive protein. Data displayed as point estimates (mean) and error bars (95% confidence intervals).

Table 4.

Overall Acute Phase Protein Levels in Postoperative Patients Undergoing Laparoscopic and Open Cholecystectomy

	Operative Approach		*P* value
	Laparoscopic	*Open*	
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 $\alpha < .05$ 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.[7,8,13–18] 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.[19–21] 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.[22] Subsequent studies on the subject has been inconclusive due to the low quality of the evidence.[23,24] 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.[22,25,26] In patients with HbAA, however, previous studies comparing open and laparoscopic cholecystectomies have highlighted immunological advantages of the laparoscopic approach.[9,27,28] 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.

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 inflammology 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.

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