Potential inflammatory targets in the integrative health care of patients with sickle cell disease

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Abstract. Inflammation plays an integral role in the complications of sickle cell disease (SCD), which can lead to vaso-occlusive crisis and extreme pain. SCD is accompanied by numerous complications, including cardiovascular disease, cognitive decline and endothelial dysfunction, contributing to mortality. As disease severity increases with age, the present study aimed to assess if age is also correlated with a definite pattern of progression of the two inflammatory markers, high-sensitivity C-reactive protein (hsCRP) and total homocysteine (tHCY). The findings of the present study could lead to an improved understanding of the threshold levels of these inflammatory markers and timely interventions to delay complications. In an observational study, levels of hsCRP and tHCY were analyzed in 70 patients (35 male and 35 female patients) with SCD aged between 5 and 16 years. hsCRP levels were in the high-risk range in 64.29% (n=45) of all male and female patients. A sex-wise distribution showed that, of the 35 male patients, 74.28% (n=26) were in the high-risk range, and of the 35 female patients, 54.28% (n=19) were in the high-risk range. An age-wise distribution showed that of the 41 patients in the 5-10-years age group, 70.73% (n=29), were in the high-risk range. In comparison, of the 29 patients in the 11-16-years age group, 55.17% (n=16) were in the high-risk range. tHCY levels were observed to be in the normal range in 98.57% (n=69) of all children, as compared with 1.43% (n=1) in the high-risk range. Furthermore, a sex-wise distribution showed that female patients in the high-risk group of hsCRP had higher concentrations of tHCY as compared with the male patients in that risk group. An age-wise distribution of hsCRP concentration also showed that the risk of CVD in patients in the 11-16-years age group was higher with increased concentrations of tHCY. A weak negative correlation was observed between age and hsCRP concentrations (r-value=-0.280; P=0.026) and a weak positive correlation was detected between tHCY and age (r-value=0.259; P=0.036). In conclusion, the results of the present study indicated that higher levels of hsCRP could be a useful marker in children with SCD, and levels of tHCY may be an adjunct marker as the disease progresses with age.

Introduction

Sickle cell disease (SCD) is a hereditary disorder, which is common in Saudi Arabia, potentially due to the traditional societal outlook and the prevalence of consanguineous marriages. SCD is typically characterized by pathophysiological complications leading to vaso-occlusive crisis (VOC) and organ damage. The etiology of SCD is multifaceted and it can result in the sickling of erythrocytes, activation of clotting due to cytokines, pulmonary infections, and cerebral complications (1).

High-sensitivity C-reactive protein (hsCRP) and total homocysteine (tHCY) are two markers that have been implicated in adverse cardiovascular events (2). CRP is an acute-phase protein, the concentration of which increases in the blood as an indicator of inflammatory disorders. A growing number of studies (3,4) have examined a more sensitive test, namely the hsCRP test, which measures lower concentrations of CRP (0.3-10 mg/l) as compared with the standard CRP test, which measures 8-1,000 mg/l concentrations. Measurement of small increases, even in the moderate range (CRP, 1-3 mg/l) for risk of CVD, is indicative of vascular inflammation, which is very useful in predicting future risk for CVD, recurrent cardiovascular disease, stroke and even death in individuals. High levels of hsCRP are implicated in patients with unstable angina, ,acute myocardial infarction and organ damage (3,4).

The role of CRP in SCD is of great interest particularly in the investigation into therapeutic solutions for this disease. When serum levels of CRP were compared between patients in the asymptomatic steady state (3.4 mg/l) and the VOC state (11.4 mg/l), a positive correlation was revealed between

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Abbreviations: hsCRP, high-sensitivity C-reactive protein; tHCY, total homocysteine; SCD, sickle cell disease; VOC, vaso-occlusive crisis

Key words: inflammation, marker, sickle cell disease

CRP levels and disease severity. The increased level of CRP (3.4 mg/l being considered high) in the asymptomatic steady state of patients with SCD seems to be protective (5). Therefore, the independent therapeutic role of CRP needs to be confirmed with larger samples in different states of inflammation in SCD (5). There seems to be an association between inflammatory biomarkers and endothelial activation in the steady state, as well as during VOC in children with SCD (6). The potential therapeutic value of CRP could be ascertained if these markers are studied in patients of different ages with SCD from childhood (6).

A previous study revealed a positive correlation between the neutrophil-to-lymphocyte ratio, and serum levels of IL-8, TNF-α and hsCRP in SCD (7). Systemic inflammation has also been reported to be associated with lower survival rates in patients with SCD and increased levels of hsCRP (8). This indicates the need for comprehensive studies to be performed on the role of inflammation in VOC and its related complications, with the aim of developing novel therapeutic strategies (9). A previous study also indicated a strong association between elevated levels of hsCRP and VOC, thus indicating its effects on VOC-related parameters and justifying the assessment of hsCRP during VOC follow-up (10,11). tHCY is another molecule that may serve a crucial role in inflammation due to its elevated levels in patients with endothelial dysfunction. Elevated concentrations of tHCY appear to be positively correlated with hospitalization due to cardiovascular disease (CVD). In addition, hospitalizations seems to be associated with older age, pre-existing CVD and lifestyle factors; therefore, the role of tHCY in inflammation in vascular disease appears to be underestimated. Higher levels in younger individuals along with inflammation should also be given due consideration (12). tHCY is a thiol-containing amino acid produced by the intracellular demethylation of methionine. tHCY represents the sum of all forms of tHCY, including the oxidized, protein-bound, and free forms. Defects in the metabolic pathway and enzymes utilizing tHCY can cause concentrations of tHCY to rise (12). This results in elevated levels of tHCY with a concurrent increased generation of free radicals, endothelial dysfunction and a decrease in nitric oxide production (13).

Patients with SCD are predisposed to ischemic complications and may therefore exhibit increased levels of tHCY, as seen in some cases (14). Notably, in general practice, children with SCD are supplemented with folate. An insufficient dietary intake of folate and B vitamins can be causative of hyperhomocysteinemia (15). Elevated levels of tHCY have emerged as an important risk factor in the assessment of CVD, and have been reported to be correlated with inflammation and immune factors, which influence the progression of numerous CVDs (16). Excess tHCY in the blood may cause injuries to arterial vessels due to their irritant nature, resulting in inflammation and plaque formation, which may eventually cause blockage of blood flow in the vascular system (17).

The current treatments commonly available for SCD are limited to administration of hydroxyurea and blood transfusions. Novel, but uncommon therapeutic options, such as oral chelation therapy, stem cell transplantation, gene therapy and gene editing are also being developed. Advanced clinical diagnostics using non-invasive MRI measurements have also been seen in modern laboratories attached to clinics (18). However, despite advancements in the treatment and diagnosis of SCD, chronic organ dysfunction and mortality are observed in these patients. Conducting research to effectively establish novel preventive and treatment options is considered a priority. The newly identified treatment options should exhibit anti-thrombotic activity and not have adverse effects of bleeding (19). Clinicians should also be made aware of the cardiovascular complications caused by COVID-19, and at the same time the necessity of blood transfusions in patients with SCD at such critical times. Patients with SCD at times require blood transfusions, and this may interfere with their cardiovascular health if they are also infected with COVID-19. Blood coagulation and endothelial damage are observed in patients infected with COVID 19; therefore, patients with SCD may have a higher risk as they already exhibit a poor immune system, and their blood cells are prone to sickling as well as related cardiovascular problems (20).

Notably, there is an urgent need for industry, health professionals, funding agencies and the government to help reduce the burden of SCD through educational awareness, and the implementation of applied research along with cost-effective interventions (21). The present study aimed to ascertain whether the levels of hsCRP and tHCY in children in a steady state with SCD in Saudi Arabia could serve as markers for the diagnosis of inflammation for earlier interventions. This study also aimed to determine whether an association exists between these inflammatory factors, and the sex and age of patients.

Materials and methods

Patient information. A prospective observational study was performed on 70 pediatric patients with SCD (age, 5-16 years) between August 2018 and December 2019 at the King Fahd Medical Research Center, King Abdulaziz University (Jeddah, Saudi Arabia). Prior to conducting the research, the protocol was approved by the King Abdulaziz University Hospital Ethics Committee, in accordance with The Declaration of Helsinki. All guardians of the subjects gave their written informed consent for their inclusion before they participated in the study, which was registered with the registration no. 2/36/8390. A control group was not assessed in the present study as informed consent could not be obtained from the parents of these children. Therefore, the criteria for sample selection depended upon the patients' visit to the hospital and their willingness to be part of the program.

Clinical steady state was defined as steady hemoglobin and hematocrit levels in the blood of the patients, along with the absence of fever, infection, VOC, or transfusion in the 4 weeks before the start of the study. VOC was defined as the patient recognizing a repeated painful episode; pain in bones, joints or multiple sites; and/or patients requiring analgesics with signs of inflammation. Patients were enrolled in a clinical steady state and blood samples were collected. Since the present study included children, the patient sample size was based on the resources available and the informed consent for participation obtained from the guardians of the patients. At 5% significance level, with a power of 80% considering a 10% standard deviation, the number of patients needed for the present study was 56. Considering dropouts and non-compliance for blood draws, 75 children were enrolled in the study, of which 70 completed the study.

Inclusion criteria. Male and female children with SCD in a steady state, aged 5-16 years old, were included in the present study. The clinical presentation of a particular genotype was defined as the phenotype. No phenotype bias was considered, and all phenotypes were enrolled. Patients with sickle cell anemia (HbSS) and sickle cell trait (HbSA) were included. The absence of infection, fever or a VOC for 4 weeks before the start of the study was taken into consideration.

Exclusion criteria. As blood draws were difficult in children <5 years of age, they were excluded from the present study. The exclusion criteria were as follows: Patients <5 years or >16 years old; obese patients with metabolic disorders; patients that had undergone blood transfusion in the last 4 weeks before enrolling for the study; the presence of other chronic diseases; patients with endocrine problems or receiving anti-coagulation treatment; previous history of overt stroke and neurological abnormalities (visual, hearing or motor deficits); chest wall anomalies or diagnoses other than VOC. In addition, all children with SCD who received hydroxyurea or folic acid treatment were excluded from the present study as this could affect their inflammatory status.

Blood collection. Venous blood (5 ml) was collected from the forearm in non-EDTA tubes, vortexed and centrifuged at 1,107 x g at 30˚C for 15 min. Subsequently, tubes were allowed to stand for 30 min prior to separating the serum obtained in the upper portion of the tubes. After labeling, the samples were stored at -80˚C.

Equipment and measurements. Serum samples (20 μl) were used for analysis of hsCRP and tHCY, which were measured using a SMART 700/340 analyzer using the automated kit method according to the manufacturers protocol. The Eurolyser SMART 700/340 multiparameter point of care analyzer (cat. no. 340/700) was obtained from Danat Al Ajiyal For Medical and Scientific Services. The standard reference range for hsCRP values was <1 mg/l, low cardiovascular risk; 1-3 mg/l, moderate cardiovascular risk; and >3 mg/l, high cardiovascular risk. The standard reference range for tHCY values was ≤14.99 μmol/l, normal; >15 μmol/l, high.

Statistical analysis. Data are presented as the mean ± standard deviation. Pearson correlation coefficient was applied using SPSS 20 (IBM Corp.) to assess the correlation between variables. P<0.05 was considered to indicate a statistically significant difference.

Results

A total of 70 patients with SCD in a steady state (age, 5-16 years) were included in the present study. Due to the lack of access to clinical data during that time due to certain hospital technicalities, the present study was unable to assess the correlation between these data and inflammatory parameters.

When hsCRP concentrations were differentiated to assess the risk of the patients for CVD, 11 children (15.71%) were found to be in the lowest risk group, with levels between 0 and 1 mg/l, and 14 children (20.00%) had a moderate risk, with levels between 1 and 3 mg/l. Even in a steady state, most of the children (n=45; 64.29%) had a level of >3 mg/l (Table I).

Table I. hsCRP levels in children with sickle cell disease (n=70).

CVD risk	hsCRP, mg/l	Number of patients (%)	Mean ± SD hsCRP, mg/l
Low	0-1	11 (15.71)	0.834±0.17
Moderate	1-3	14 (20.00)	1.91±0.63
High	>3	45 (64.29)	8.56±5.12

CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein.

tHCY levels were observed to be normal in 98.57% (n=69) of all children, as compared with 1.43% (n=1) who showed a very high concentration (Table II).

A sex-wise distribution for hsCRP concentrations showed that, of the 35 male patients, 74.28% (n=26) were in the high-risk range, and of the 35 female patients, 54.28% (n=19) were in the high-risk range (Table III). A sex-wise distribution showed that female patients in the high-risk CVD group had higher concentrations of tHCY (8.05±2.55 μmol/l) as compared with the male patients (7.43±2.84 μmol/l) in that risk group (Table III). An age-wise distribution showed that, of the 41 patients in the 5-10-years age group, 70.73% (n=29) were in the high-risk range for CVD. In comparison, of the 29 patients in the 11-16-years age group, 55.17% (n=16) were in the high-risk range for CVD (Table IV). Most of the children in the 5-10-years age group (n=29) exhibited high hsCRP levels, with a mean concentration of 9.29±5.56 μmol/l whereas those (n=16) in the 11-16-years age group (n=16) showed a mean of 7.34±4.17 mg/l (Table IV).

An age-wise distribution also showed that in all the risk groups of CVD, patients in the 11-16-years age group had higher concentrations of tHCY when compared with children in the 5-10-years age group (Table IV). One of the female patients in the 11-16-years age group showed a very high tHCY level of 22.56 μmol/l.

As both inflammatory markers have also been implicated in vascular diseases, the present study aimed to identify any association between tHCY levels and the different risk levels of CVD. Of the two age groups, tHCY concentration in the 11-16-years age group was higher (Table II) with one female patient in the high CVD risk group showing a very high concentration of tHCY. Incidentally, this patient was also the oldest girl in the group. The numbers were small enough and unequal in the two age groups, which prevented further statistical analysis. Larger numbers would give a better picture.

Although weak, a negative correlation was observed between age and hsCRP concentrations (r=-0.280; P=0.026), whereas a weak positive correlation was observed between age and tHCY (r=0.259; P=0.036) (Table V).

Discussion

Being a strong predictor of morbidity due to cardiovascular manifestations, the levels of hsCRP in children with SCD

Table II. tHCY levels in children with sickle cell disease.

	All patients (n=70)		Male patients (n=35)		Female patients (n=35)		Patients aged 5-10 years (n=43)		Patients aged 11-16 years (n=27)	
tHCY	Number of patients (%)	tHCY levels, μmol/l	Number of patients (%)	tHCY levels, μmol/l	Number of patients (%)	tHCY levels, μmol/l	Number of patients (%)	tHCY levels, μmol/l	Number of patients (%)	tHCY levels, μmol/l
0-14.9 μmol/l	69 (98.57%)	7.20±2.97[a]	35 (50.00)	6.98±2.99[a]	34 (48.57)	7.42±2.99[a]	43 (61.43)	6.69±3.03[a]	26 (37.14)	7.96±2.78[a]
≥15 μmol/l	1 (1.43)	22.56	0 (0.00)	-	1 (1.43)	22.56	0 (0.00)	-	1 (1.43)	22.56

[a]Mean ± SD. tHCY, total homocysteine.

needs to be monitored carefully, since they could signal a cardiovascular problem or other complications with which these children are generally affected. Studies have shown that both hsCRP and tHCY have independently emerged as predictive risk factors of CVD (4,13). Although the pathways of hsCRP and tHCY are independent of each other, concentrations of both seem to contribute towards a high incidence of severe inflammatory response in the body, with an increased number of unhealthy arteries, thereby leading to coronary artery disease (2).

Levels of hsCRP have been shown to be significantly higher in children with SCD, which is suggestive of chronic inflammation in these patients. Since hsCRP is a potential inflammatory mediator, its association with VOC is of great significance (10). Chronic inflammation during the steady state of SCD thereby justifies in part the adherence of sickle erythrocytes to the endothelium, which could also be due to increased cholesterol levels. Adherence of erythrocytes to the endothelium promotes thrombosis, which is seen in all children with SCD (22). A similar inflammation was also observed in the present study, with the high-risk group for CVD having higher concentrations of hsCRP and tHCY in the steady state. In another study on children with SCD from Saudi Arabia, children aged 4-13 years (mean ± SD, 10.73±2.1 years) exhibited hsCRP levels of 4.14±1.29 mg/l, compared with 1.65±0.82 mg/l for healthy controls without SCD (23).

Notably, age seems to be a contributory factor in the progression of inflammation and disease severity. A negative correlation between age and hsCRP indicates improved management of the disease, as age progresses as observed in previous studies. It has been observed that tHCY levels appear to vary slightly amongst different ethnic groups and ages. In a study from India, the mean tHCY concentration was 8.7±4.25 μmol/l in a healthy control group of children without SCD (age, 11.7±8.2 years) (24). tHCY levels for healthy controls without SCD aged 8.85±2.66 years was found to be 6.32±1.47 μmol/l in Egyptian children (8) and 7.5±0.3 μmol/l for Greek school children aged 6-15 years (25). Adult patients with SCD exhibit inflammation during steady-state conditions, which is reflected by increased hsCRP levels. In patients with HbSS, CRP has been reported to be strongly and independently associated with increased mortality (26). It has been reported that in patients with SCD, age appears to be an independent predictor of microvasculopathy, and that the administration of appropriate vitamin B supplements during childhood could potentially decrease the associated complications observed (27). In the present study, when levels of tHCY were considered, almost all children with SCD (98.57%) exhibited normal concentrations, with 1.42% (n=1) of patients in the 11-16-years age group exhibiting concentration in the higher range. Nevertheless, when considering the 11-16-years age group, all of the 16 children in the higher risk hsCRP range had higher concentrations of tHCY, with one female patient showing a very high mean concentration of tHCY. Advancement of the disease as age progresses could be one of the reasons. The observations of this study could give us leads for future research. Although the levels of tHCY detected in the present study were slightly higher than those observed in previous studies (8,25), most fell within the normal range. Decreases in the activity of glutathione selenoperoxidase have been implicated in atherosclerosis. This enzyme is

Table III. Sex-wise distribution of hsCRP and tHCY based on the level of risk for CVD.

A, Male patients (n=35)

Level of risk	hsCRP, mg/l	Number of patients (%)	Mean ± SD hsCRP, mg/l	Mean ± SD tHcy, μmol/l
Low	0-1	3 (8.57)	0.86±0.05	3.55±0.49
Moderate	1-3	6 (17.14)	1.75±0.74	6.91±3.46
High	>3	26 (74.28)	9.29±5.56	7.43±2.84

B, Female patients (n=35)

Level of risk	hsCRP, mg/l	Number of patients (%)	Mean ± SD hsCRP, mg/l	Mean ± SD tHcy, μmol/l
Low	0-1	8 (22.86)	0.821±0.20	9.39±5.79
Moderate	1-3	8 (22.86)	2.03±0.53	6.03±4.05
High	>3	19 (54.28)	7.34±4.17	8.05±2.55

hsCRP, high-sensitivity C-reactive protein; tHCY, total homocysteine.

Table IV. Age-wise distribution of hsCRP and tHCY based on the level of CVD risk.

A, Patients aged 5-10 years (n=41)

Level of risk	hsCRP, mg/l	Number of patients (%)	Mean ± SD hsCRP, mg/l	Mean ± SD tHCY, μmol/l
Low	0-1	4 (9.76)	0.86±0.05	4.83±1.39
Moderate	1-3	8 (19.51)	1.93±0.61	5.30±3.31
High	>3	29 (70.73)	9.29±5.56	7.26±2.94

B, Patients aged 11-16 years (n=29)

Level of risk	hsCRP, mg/l	Number of patients (%)	Mean ± SD hsCRP, mg/l	Mean ± SD tHCY, μmol/l
Low	0-1	7 (24.14)	0.83±0.25	9.49±6.44
Moderate	1-3	6 (20.69)	2.383±0.55	7.88±3.95
High	>3	16 (55.17)	7.34±4.17	8.65±2.04

hsCRP, high-sensitivity C-reactive protein; tHCY, total homocysteine.

responsible for preventing oxidative damage and protecting the endothelium from atherogenesis. Age-related changes have also been observed in the glutathione redox system making it more vulnerable to oxidative damage as age progresses (28). In a study conducted across different age groups, although selenium levels were not significantly altered in children and adults a selective loss of glutathione peroxidase 1 in healthy old subjects was detected, which could trigger a loss of antioxidant defense. This in turn could decrease the capacity to deal with ischemic injury (29). tHCY appears to inhibit the activity of the protective enzyme glutathione selenoperoxidase (30), thereby potentially compounding cardiovascular problems. This could justify why physiological concentrations of tHCY could be associated with CVD (30). In the present study, a weak positive correlation was detected between age and tHCY in children with SCD, thus indicating their vulnerability to cardiovascular problems later in life. The levels of hsCRP in pediatric patients in a steady state from other studies show that the inflammatory status is increased in children with SCD at an early age (9). The inflammatory marker hsCRP was also increased in all children with SCD, even though they were in a steady state in

Table V. Correlation between hsCRP and tHCY concentrations and the age of patients.

Correlation	Pearson correlation coefficient, r-value	P-value
Age with hsCRP	-0.280	0.026
Age with tHCY	0.259	0.036

hsCRP, high-sensitivity C-reactive protein; tHCY, total homocysteine.

the present study as compared with the normal concentration in their healthier counterparts. The normal levels of tHCY in pediatric patients in other studies is comparable to the levels observed in the present study on children with SCD; however, in the present study, as age progressed, the value of tHCY exhibited an increase. Thus, an increase in tHCY could be an indicator of disease progression.

It has also previously been hypothesized that the concentrations of folic acid required to normalize levels of tHCY in patients with SCD may be greater than in normal children (31). A previous study showed that tHCY, along with other inflammatory molecules, such as IL-17 and soluble vascular adhesion molecule, seem to contribute to VOC, which affirms its role in inflammation (32). Consistently maintaining normal tHCY values low may decrease the morbidity and mortality associated with the aging process (33). A previous study reported that tHCY concentrations appear to be a strong risk factor for VOC and other complications in older children (34). In the present study, the female cohort in both age groups showed higher levels of tHCY. In the present study, although normal concentrations of tHCY were observed in almost all of the patients in the 11-16-years age group, the mean concentration of tHCY was higher when compared with the mean concentration of tHCY in the patients in the 5-10-years age group, which is indicative of a comparatively increased risk for various clinical complications. When compared with male patients, the female patients exhibited slightly higher tHCY concentrations (although within normal limits), which has also been reported in a previous study in patients with SCD aged 6-11 years (35). A previous meta-analysis indicated that elevated tHCY levels are linearly associated with physiological dysfunctions in a dose-dependent manner, with each 5-μmol/l increase in tHCY causing a 33.6% increase in the risk for major chronic diseases, such as CVD, cognitive decline, and endothelial dysfunction, contributing to all-cause mortality (27). It has been suggested that the administration of appropriate vitamin-B supplements during childhood could decrease the associated complications observed in SCD (27). Although folate is usually supplemented to children with SCD, the prescribed dose may be insufficient to combat the augmentation of inflammation and processes that increase tHCY concentrations. By contrast, a previous study suggested that the increases in tHCY are irrespective of the vitamin B12 and folate levels with which tHCY is usually associated with in SCD (36). Another study suggested that mild hyper homocysteinemia may promote atherogenesis by inducing leukocyte infiltration to the site of vascular injury (37).

In conclusion, the present study suggested that although both hsCRP and tHCY concentrations are higher in the steady state, only tHCY levels may be elevated at later stages (higher age group) as the diseases progresses. Although only one patient showed an increase in tHCY concentration, the patient was in the high-risk for CVD (hsCRP) group, which consisted of only 16 children in that age group. Patients in the high risk for CVD group showing higher concentrations of tHCY should therefore be examined carefully for cardiovascular problems. The percentage of patients in this group may be few; however, when larger studies are conducted, this could possibly encourage clinicians to necessitate the analysis of tHCY in children with SCD >11 years old to prevent these patients from cardiovascular problems early in life. The results indicated that elevated hsCRP levels, observed in a steady state in patients with SCD, may contribute towards vascular inflammation and endothelial dysfunction (9). This could be considered important in the pathogenesis of SCD, paving the way for novel prognosis by clinicians (3).

In addition, as tHCY concentrations appear to increase in the older age group, a reconsideration of folate and vitamin B12 supplementation dosages is warranted. Future elaborative studies are required with a larger number of pediatric patients of varying ages, exploring the roles of tHCY and hsCRP at different stages of the disease. These studies could identify adjunct markers in the assessment and therapeutic targeting of SCD. The present data highlights the medical relevance of these inflammatory markers in children with SCD and provides a basis for future therapeutic strategies.

The present study has some limitations. A larger sample size, and a control group, which could not be accomplished in the present study, are warranted, to further establish the role of inflammatory markers in SCD. In addition, unequal distribution of male and female patients in the different age groups prevented the performance of appropriate statistical tests. Furthermore, due to the lack of clinical data availability, the correlation between inflammatory status and clinical characteristics, such as blood profile and blood pressure, could not be assessed.

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Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to confidentiality reasons, but are available from the corresponding author on reasonable request.

Authors' contributions

ShAK created and designed the study. ShAK, SHH, TAZ and SaAK performed data collection and analysis. ShAK and SHH interpreted the data. ShAK, SHH, TAZ and SaAK wrote the article. All authors confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate.

Prior to conducting the research, the protocol was approved by the Ethics Committee of King Abdulaziz University Hospital, in accordance with the Declaration of Helsinki. All guardians of the subjects gave their written informed consent for inclusion before they participated in the study (registration no. 2/36/8390).

Patient consent for publication

All guardians of the subjects gave their consent for publication of the results. Written informed consent was obtained for the use of their data and results.

Competing interests

The authors declare that they have no competing interests.

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