



## ORIGINAL ARTICLE

# Omega 3 fatty acids - Potential modulators for oxidative stress and inflammation in the management of sickle cell disease



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### Abstract

**Objective:** Sickle cell disease is characterized by clinical complications resulting in vaso-occlusive crisis with prominent attributes of oxidative stress, inflammation, and pain. Inflammation is an integral part of this disease which further exacerbates the pain during a crisis. Omega-3 fatty acids are known to possess anti-inflammatory and anti-aggregatory properties and assist in diminishing the slow physiological inactivation.

**Methods:** A pilot nutritional interventional study was conducted wherein forty-three children with sickle cell disease aged 5-16 years were supplemented with omega-3 fatty acids for a period of six months. Analysis of oxidative stress, as well as inflammatory parameters, was done pre and post-supplementation.

**Results:** Increased free oxygen radical transference values depicting free radical generation is enhanced in these patients along with a reduced antioxidant defense, as seen by decreased free oxygen radical defense values. Supplementation with omega-3 fatty acids for a period of six months significantly reduced the inflammatory marker homocysteine in all patients, whereas high sensitive C reactive protein was significantly reduced only in females of the age group 11-16years. Simultaneously a significant reduction in oxidative stress parameters with a concomitant increase of antioxidant defense was observed in all patients.

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**Conclusion:** The authors' findings suggest the regulatory effects of omega-3 fatty acids as cellular activators in alleviating the complications due to sickle cell disease. Omega-3 fatty acids hold promise as future therapeutic candidates in patients with sickle cell disease.

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## Introduction

Sickle cell disease (SCD) typically features a painful hemolytic crisis, which leads to exacerbated inflammation and oxidative stress.<sup>1,2</sup> These sickled red blood cells (RBCs) and their hemolysis ultimately contribute to the continuing vaso-occlusion at varying short intervals, leading to a pro-inflammatory state, releasing reactive oxygen species (ROS). Continual stress and chronic inflammation seem to be an integral part of SCD. Mechanisms of persistent pain in SCD are yet to be properly understood. Apart from analgesics and anesthetics, opioids are used to manage the pain. But opioids are known for their side effects of dependency, hyperalgesia and adversely influencing RBC cell structure and its properties.<sup>3</sup> High sensitive C reactive protein (hsCRP) is known to be a significant contributor, and low-grade inflammatory marker implicated in the progression of vaso-occlusive crisis (VOC) in pediatric patients. Baseline levels of hsCRP were found to be increased in SCD children, and this correlated with higher demands of energy even during the resting state.<sup>4</sup> An elevated level of total homocysteine (tHcy) has in recent times emerged as an important risk factor for the assessment of cardiovascular problems. Its increase results in inflammation and plaque formation, which may eventually cause blockage of blood flow to the heart.<sup>5</sup>

The amplification of oxidative status in SCD is independent of age, genotype, and therapy, according to a previous study.<sup>6</sup> It was also observed that an uncontrolled production of free radicals frequently leads to an impairment of cellular protein, DNA, and lipids along with a few small antioxidant molecules.<sup>7</sup>

SCD leads to continuous production of ROS accompanied by an increase in the auto-oxidation of the red blood cells. The levels of antioxidants seem to be compromised in SCD.<sup>8,9</sup> The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exhibit anti-inflammatory properties, and their inclusion in food is known to reduce inflammatory cell processes. Increases in the omega-6 fatty acid (arachidonic acid) appear to be directly proportional to the decreases in EPA & DHA, which in turn increases levels of hsCRP. Omega-3 fatty acids are known to accelerate positive changes in the RBC membrane of the sickle cell by enhancing the production of nitric oxide, reducing inflammation and coagulation.<sup>10</sup> They act as precursors to the formation of anti-inflammatory and anti-aggregatory bioactive lipids, namely, resolvins, protectins and lipoxins.<sup>11</sup> They exhibit direct or indirect suppression in the activity of nuclear transcription factors, NF $\kappa$ B, and reduction in the production of cytokines including COX-2, tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and pro-inflammatory enzymes, which induce pain and fever.<sup>4</sup> The diverse role of omega-3 fatty

acids makes them promising therapeutic candidates for the prevention of sickling and reduction of the painful crisis in sickle cell disease.<sup>12</sup>

The authors of the present study therefore aimed at reducing the dependence of these SCD pediatric patients on treatments that may have long-term side effects and pain. The authors hypothesized that a model shift to a concurrent nutritional intervention could delay the progression of sickling and thereby reduce the painful episodes in the Saudi Arabian pediatric population.

## Methods and materials

A pilot nutritional interventional study with omega-3 fatty acids was conducted at King Fahd Medical Research Center, King Abdulaziz University, Jeddah, for a period of six months after appropriate approval by the institutional ethical committee (registration no-2/36/8390).

### Criteria for sample selection

While selecting samples for the present study, practicality and inflow of patients (aged 5-16 years) from the hospital follow up clinic or daycare were considered.

### Sample size

Information on the expected favorable clinical changes after six months of omega-3 fatty acid supplementation was obtained from the study reported by Daak and associates<sup>12</sup> and the pilot study by Okpala and co-authors.<sup>13</sup> Accordingly, the sample size needed to be able to detect a 25% improvement in VOC at a 5% significance level, with a power of 80% and 10% standard deviation was  $n = 56$  in each group. The desired number of patients could not be obtained due to non-compliance. With 210 patients that were identified and screened, a total of 155 patients were found to be suitable to the authors' criteria, of which 110 gave their consent, but only 50 of them were available during blood collection and supplementation. Of these 43 children could complete pre and post supplementation blood withdrawals. Since the authors were dealing with children, the study's patient sample size was therefore mainly based on all the resources available, as well as the acceptance of the patient's guardians for participation.

### Patient demographics

Forty-three pediatric patients with SCD aged 5-16 yrs who agreed to participate were enrolled for the supplementation study. All phenotypes were enrolled. A placebo or control

group could not be taken for the study due to non-compliance. Therefore, their pre supplementation values served as their own controls.

**Inclusion criteria:** Pediatric male and female SCD patients, in a steady-state, and aged between 5-16 years were enrolled. The absence of fever, infection, or crisis for longer than four weeks before the start of the study was taken into consideration.

**Exclusion criteria:** Patients much above the average weights, unmatched for age; undergone transfusion within the past 10 weeks before enrolling for the study; those on hydroxyurea and diagnosed with chronic problems other than VOC, were excluded.

### Interventions

All children enrolled were supplemented with mango flavored omega-3 syrup from BRAINWISE Co., SPB Laboratories, HP India, for a period of six months. Patients with body weights 11-24 Kg were supplemented with 2 teaspoons of syrup per day and those with body weights 25Kg and above were given 3 teaspoons of syrup per day. One teaspoon of the syrup consisted of 190 mg DHA, and 250 mg EPA. As hydroxyurea (HU) usage may affect VOC and the assessed parameters, therefore only patients who were not on hydroxyurea were recruited for omega-3 supplementation. This study was conducted with the intention to treat the said population. Compliance with the supplement was assessed by considering 80% consumption of syrup every month.

### Blood collection

Five ml of venous blood was drawn, and depending upon the assay, either the heparinized blood or serum samples were taken for the assay.

### Oxidative stress parameters

**Free Oxygen Radical Transference (FORT) assay**

20 µL of heparinized blood sample was taken, processed, and analyzed according to the kit method (Callegari SpA, Catellani Group, Parma, Italy).<sup>14</sup>

**Free Oxygen Radical Defense (FORD) assay**

50 µL heparinized blood samples were taken to analyze total antioxidant capacity FORD using standardized kits.<sup>15</sup>

### Analysis of inflammatory markers

**Analysis of Hs CRP**

20 µL serum was taken for analyzing HsCRP with a SMART 700/340 analyzer using the automated kit method.<sup>16,17</sup>

**Analysis of homocysteine**

20 µL serum was taken for analyzing total HCY by the Eurolyzer SMART 700/340 instrument using a standard kit method.

### Statistical methods

All obtained data was processed and expressed as means ± the standard deviations. Statistical analysis was performed using SPSS 21.0 version. Paired t-test was performed, and p values of < 0.05 were considered significant.

### Results

Out of the 43 enrolled patients, 23 were males and 20 were females. Fifteen males were in the age group 5-10 years and eight were in the age group 11-16 years. Eleven females were in the age group 5-10 years and nine were in the age group 11-16 years. In all, a total of twenty-six children were in the 5-10 age group (all in the range 11-24 Kg body weight) and seventeen children were in the 11-16 age group (≤ 25 kg body weight). The mean weight of children aged 5-10 years was 19.89 Kg with SD 5.7, and the mean weight of children aged 11-16 years was 33.98 Kg with SD 8.85.

All patients enrolled were of HbSS genotype. Due to insufficient blood collections from 3 male children belonging to the age group 5-10 years, FORT and FORD analyses were performed in only 40 patients. No adverse effects were observed during the entire supplementation period of six months. No bleeding events were noticed or reported. The only minor complaint received at the beginning of the study was the slight gastric upset experienced by 3 children, which eventually subsided in 2-3 days.

Oxidative stress was measured in terms of FORT values denoting the free oxygen radical transference capacity and FORD values denoting the antioxidant defense capacity, which are depicted in Tables 1, and 2, respectively. All values observed for FORT were higher than the normal range prior to supplementation (4.029 ± 0.467), denoting increased stress. The oxidative stress was reduced significantly after omega-3 supplementation (3.286 ± 0.872) with a 'p-value of 0.000, as seen in Table 1. Males of both age groups showed a significant reduction in FORT with 'p' values

**Table 1** Free oxygen radical transference (FORT) of SCD patients before and after omega-3 supplementation.

Parameter	n	PRE (mean ± SD)mmol/L H <sub>2</sub> O <sub>2</sub>	POST (mean ± SD)mmol/L H <sub>2</sub> O <sub>2</sub>	p value
FORT	40	4.029 ± 0.467	3.286 ± 0.872	.000
Male 5-10 years	12	4.077 ± 0.558	3.356 ± 0.905	.000
Male 11-16 years	8	3.713 ± 0.522	2.883 ± 0.411	.002
Female 5-10 years	11	3.975 ± 0.478	3.325 ± 1.010	.000
Female 11-16 years	9	4.046 ± 0.393	3.366 ± 0.959	.005

(Normal range for FORT --- 1.75-3.04 mmol/L H<sub>2</sub> O<sub>2</sub>).

Values above (above 3.04 mmol/L H<sub>2</sub> O<sub>2</sub>) are considered as exhibiting high oxidative stress, between (1.75-3.04 mmol/L H<sub>2</sub> O<sub>2</sub>) as intermediate, and values below (1.75 mmol/L H<sub>2</sub> O<sub>2</sub>) as exhibiting reduced stress.

**Table 2** Free oxygen radical defense (FORD) of SCD patients before and after omega-3 supplementation.

Parameter	n	PRE (mean ± SD)mmol/L Trolox	POST (mean ± SD)mmol/L Trolox	p value
FORD	40	.907 ± 0.437	1.518 ± 0.642	.000
Male 5-10 years	12	0.854 ± 0.425	1.216 ± 0.28	.000
Male 11-16 years	8	.995 ± .505	1.704 ± .706	.004
Female 5-10 years	11	1.083 ± .563	1.210 ± .394	.000
Female 11-16 years	9	0.928 ± 0.553	1.880 ± 0.798	.006

(Normal range for FORD -- 1.07-1.53 mmol/L Trolox).

Values above 1.53 mmol/L Trolox are considered as good antioxidant capacity, between 1.07 and 1.53 mmol/L Trolox- normal and values below 1.07 considered as reduced antioxidant ca.

0.000 and 0.002, respectively. Likewise, females of both age groups showed a significant reduction in FORT with 'p' values 0.000 and 0.005, respectively.

Table 2 represents data for free oxygen radical defense values of all patients enrolled for supplementation. FORD values were either reduced or on the lower side of the normal range in all patients ( $0.907 \pm 0.437$ ), which increased after supplementation to ( $1.518 \pm 0.6423$ ) with a significance of 0.000. Both males and females showed a significant increase in FORD values after supplementation with 'p' values of 0.000 and 0.004 in the different age groups of males; and 0.000 and 0.006 in the two age groups of females, respectively.

The levels of the inflammatory markers hsCRP and HCY of the patients are depicted in Tables 3 and 4, respectively. Though not statistically significant, levels of the inflammatory marker hsCRP were reduced from  $7.4344 \pm 7.2822$  to  $6.4349 \pm 5.7199$  after supplementation with a 'p' value of 0.363 as seen in Table 3. According to the gender, although there was a reduction in the levels of the inflammatory markers hsCRP in both males and females, however, the differences were not statistically significant. In addition, it is important to note that the level of hsCRP in females of the 5-10 age group was decreased markedly, however, it did not achieve significance. Nevertheless, the hsCRP levels in females of the 11-16 age group decreased significantly with a p-value of .007.

Levels of the other inflammatory marker homocysteine were found to decrease significantly from  $7.200 \pm 2.586$  to  $4.544 \pm 2.313$  after supplementation with a 'p-value of 0.000, as observed in Table 4. Males, as well as females of both age groups, showed significant reductions in homocysteine levels.

Males of both age groups showed statistically significant 'p' values of 0.000 and females showed 'p' values of 0.000 and 0.021 for the two age groups 5-10yrs and 11-16 yrs respectively.

## Discussion

Although much research on SCD has been done in Saudi Arabia, this is probably the first omega-3 interventional study on oxidative stress and inflammation in children with SCD. Several studies associate SCD with an overload of reactive pro-oxidant products, which may be responsible for the decrease in antioxidant defense.<sup>18</sup> As noticed by some researchers, the endogenous antioxidant defense mechanism may not be very effective.<sup>19</sup>

In the present study, the authors observed that omega 3 supplementation decreased oxidative stress, with significantly lowered FORT. Previous studies showed that DHA prevented atherosclerosis by increasing the monocyte resistance to LDL (oxidized) induced apoptosis. Furthermore, it regulates the endothelial cell function as observed earlier.<sup>20,21</sup> This antioxidant defense system scavenges the free radicals, lowers immune dysfunction, and decreases metabolic disturbances.

It has been observed that patients with SCD showed decreased levels of total antioxidant and increased levels of total oxidative stress even in the steady-state. The sickled HbS- red cells experience faster auto-oxidation generating more lipid peroxidation products and superoxides, as compared to the normal HbA cells. A significant correlation exists between the disease severity and oxidative stress markers.<sup>22</sup> Supplementation of omega-3 fatty acids is known

**Table 3** Hs CRP levels in SCD patients pre and post omega-3 fatty acids supplementation.

PARAMETER	n	PRE (mean ± SD)mg /L	POST (mean ± SD)mg /L	p value
HsCRP	43	7.434 ± 7.282	6.4349 ± 5.719	.363
Male 5-10 years	15	9.940 ± 6.507	9.064 ± 5.578	.637
Male 11-16 years	8	4.811 ± 6.491	3.031 ± 2.001	.338
Female 5-10 years	11	5.211 ± 7.259	3.469 ± 4.393	.073
Female 11-16 years	9	12.193 ± 8.443	3.578 ± 1.670	.007

Reference range:

- < 1 mg/L - low cardiovascular risk.
- < 1-2mg/L - moderate cardiovascular risk.
- > 3mg/L - high cardiovascular risk.

**Table 4** Homocysteine levels in SCD patients before and after omega 3 fatty acid supplementation.

Parameter	n	PRE (mean ± SD) μmol of tHCY /L	POST (mean ± SD) μmol of tHCY /L	p value
Homocysteine	43	7.200 ± 2.586	4.544 ± 2.313	.000
Male 5-10yrs	15	7.622 ± 2.509	3.340 ± 2.074	.000
Male 11-16yrs	8	6.185 ± 1.801	4.195 ± 1.055	.000
Female 5-10yrs	11	6.061 ± 1.538	2.907 ± 0.177	.000
Female 11-16yrs	9	8.474.122 ± 2.541	5.370 ± 1.316	.021

The standard normal reference range considered was 5-15 μmol of tHCY /L

to improve microvascular function and exhibit significant decreases in oxidative stress, as well as inflammation, thereby reducing the pain episodes and improving the patient’s quality of life.<sup>23</sup> Earlier studies have shown that congenital mutations play a role in unbalancing the total antioxidant and free radical generation status. Supplements halting this vicious cascade hold promise to children with SCD. Therefore, interventions aiming at increasing the antioxidant potential could possibly benefit such patients.<sup>24</sup> As of now though HU remains the drug of choice, it seems to only partially contribute to alleviating oxidative stress.<sup>25</sup>

Earlier data showed a more than 53% decrease in VOCs among patients treated with omega-3 fatty acid DHA. It is reported that the abnormalities of membrane fatty acids, their rheology, inflammatory status, and hemolysis were improved with omega-3 fatty acids treatment. The multidimensional pleiotropies of omega-3 fatty acids could possibly prove in the future to be an effective, safe, and affordable therapy for the disease.<sup>26</sup>

hsCRP has been implicated as an important biomarker for assessing systemic inflammation and microvessel occlusion, which may lead to a painful crisis in children. The results in the present study showed elevated levels of hsCRP in all patients prior to supplementation as seen in Table 3. Although not significant, hsCRP levels decreased in all children who had experienced more occlusive pain at the beginning of the study. It has been suggested that omega-3 intake may provide a greater reduction in the risk of cardiovascular complications in such patients. Therefore, if the risk factor for these patients towards cardiovascular problems is considered, then the observed favorable changes seem appreciable. Earlier research has shown strong associations between hsCRP, time of hospitalization and adverse clinical health outcomes. In agreement with the authors’ findings, there are several studies that reported that the increased pain and the levels of hsCRP are well correlated.<sup>27</sup>

Endothelial dysfunctions have been known to lead to alterations in homocysteine levels in SCD patients. Mildly increased HCY levels are observed in about 20% of the older patients despite the normal folate and cobalamin status. The values are varied depending upon the geographical influences and age of the child, as noted in earlier studies. This seems to be associated with creatinine levels which are expressed eventually as renal dysfunction in many SCD patients.<sup>28</sup>

In the present study, supplementation with omega-3 fatty acids reduced tHCY levels in both genders, as well as age groups with significant ‘p’ values. The mean tHCY levels in

the present study were normal at the start of the study. However, they were found to be on the higher side of the normal values for this age group of children, which is similar to the findings reported in earlier studies.<sup>29</sup> In addition, this observation has been suggested to be responsible for the hypercoagulability and the resulting thromboembolic complications.<sup>30</sup> A further reduction in an inflammatory marker may contribute to combating infections and other adverse effects observed in this disease. Moreover, the minor side effects with omega-3 fatty acids being a gastric disturbance in a few, would not be a deterrent for its usage for longer periods of time.

### Limitation

Further studies with a control group, larger sample size, and longer time period are warranted to establish the therapeutic influence of omega-3 fatty acids in SCD.

The present study’s results showcase the multifaceted approach of omega-3 fatty acids, making them a potentially safe and affordable therapy, targeting dual markers of oxidative stress as well as inflammation in SCD. Future trials on a larger scale are warranted to exploit this potential of omega-3 fatty acids further.

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### Conflicts of interest

The authors declare no conflicts of interest.

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