Effect of omega-3 (n−3) fatty acid supplementation in patients with sickle cell anemia: randomized, double-blind, placebo-controlled trial[1–3]

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ABSTRACT

Background: Blood cell aggregation and adherence to vascular endothelium and inflammation play a central role in vaso-occlusive crisis in sickle cell disease. The antiaggregatory, antiadhesive, anti-inflammatory, and vasodilatory omega-3 (n−3) fatty acids (DHA and EPA) are significantly reduced in patients with the disease.

Objective: The aim was to investigate the therapeutic potential of omega-3 fatty acids for patients with homozygous sickle cell disease in a randomized, placebo-controlled, double-blind trial.

Design: One hundred forty patients recruited from a single center in Sudan were randomly assigned and received, daily, 1 (age 2–4 y), 2 (age 5–10 y), 3 (age 11–16 y), or 4 (age ≥17 y) omega-3 capsules containing 277.8 mg DHA and 39.0 mg EPA or placebo for 1 y. Of these patients, 128 were followed up and the data were obtained. The primary and secondary endpoints—rates of clinical vaso-occlusive crisis and hemolytic events, blood transfusion rate, school attendance, and blood count—were analyzed by intention-to-treat analysis (n = 140).

Results: Omega-3 treatment reduced the median rate of clinical vaso-occlusive events (0 compared with 1.0 per year, $P < 0.0001$), severe anemia (3.2% compared with 16.4%; $P < 0.05$), blood transfusion (4.5% compared with 16.4%; $P < 0.05$), white blood cell count (14.4 ± 3.3 compared with 15.6 ± 4.0 $\times 10^3/\mu$L; $P < 0.05$), and the OR of the inability to attend school at least once during the study period because of illness related to the disease to 0.4 (95% CI: 0.2, 0.9; $P < 0.05$).

Conclusion: The findings of this trial, which need to be verified in a large multicenter study, suggest that omega-3 fatty acids can be an effective, safe, and affordable therapy for sickle cell anemia. This trial was registered with Current Controlled Trials as ISRCTN80844630. *Am J Clin Nutr* 2013;97:37–44.

INTRODUCTION

Sickle cell disease is a group of autosomal recessive genetic blood disorders characterized by a single point mutation in the sixth codon of the β-globin gene. Under low oxygen tension, the resultant abnormal hemoglobin S polymerizes and causes rigid and sickle-shaped red blood cells (1). Homozygous sickle cell disease (HbSS)[4], also known as sickle cell anemia, is the major and severest form (2). In sub-Sahara Africa, the prevalence of sickle cell trait ranges between 5% and 40% (3), and >230,000 (0.74% of total birth) infants are born with sickle anemia every year (3, 4). Vaso-occlusive crisis is the main clinical manifestation and cause of hospitalization, organ damage, and death (5–7). Moreover, stroke is the most serious complication and the major cause of physical disability and cognitive impairment (8, 9). More than 10% of patients with sickle cell anemia develop overt stroke, and ∼22% show evidence of silent cerebral infarction (10, 11). In Africa, the life expectancy of patients with sickle cell disease is <20 y (12), and those <5 y of age are at the highest risk of death (13).

It was thought that vaso-occlusive crisis is caused by a mechanical obstruction of small blood vessels by rigidly distorted (sickled) red blood cells (14). However, there is no relation between the number of irreversible sickled cells and vaso-occlusive crisis (15, 16). However, the blood cells of patients with sickle cell disease have a tendency to adhere to vascular endothelium, and there is a correlation between blood cell–vessel wall adhesive interactions and vaso-occlusive crisis (17–19). These findings have led to the current postulation that an enhanced tendency of red blood cells (sickled and nonsickled) to adhere to vascular endothelium and activation of platelets and leukocytes are the primary causative factors of vaso-occlusion (20–22). The aforementioned factors are strongly modulated by cell membrane PUFAs (23–28). Indeed, patients with steady state sickle cell disease have abnormal red cell, platelet, and mononuclear cell PUFA composition characterized by elevated arachidonic acid (AA, 20:4n−6), adrenic acid (22:4n−6), and osbond acid (22:5n−6) and decreased linoleic acid (LA, 18:2n−6), EPA (20:5n−3), and DHA (22:6n−3) (29, 30). This suggests that the

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[4] Abbreviations used: AA, arachidonic acid; HbSS, homozygous sickle cell disease; LA, linoleic acid; MCH, mean corpuscular hemoglobin.

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abnormality of blood cell membrane PUFAs may contribute to the disregulation of blood cell–vessel wall interaction and vaso-occlusive crisis in patients with sickle cell disease. It has been reported that supplementation with fish oil containing omega-3 (n−3) fatty acids (EPA and DHA) reduces the frequency of pain episodes requiring hospital presentation (31) and the number of sickle cell crises (32). The latter did not use placebo controls, and both studies were not sufficiently powered to assess unbiased outcomes. Therefore, the aim of this double-blind, placebo-controlled trial was to investigate the therapeutic potential of omega-3 fatty acid supplementation in patients with HbSS.

SUBJECTS AND METHODS

Patients

Patients aged 2–24 y with HbSS, who were undergoing regular follow-up at the outpatient Sickle Cell Disease Referral Clinic, Ibn-Aoaf Paediatric and Khartoum Teaching Hospitals, Khartoum (Sudan), were enrolled between April 2009 and May 2010. The patients were in a steady state, defined as no evidence of fever, infection, or crisis for >4 wk before the start of the study. Phenotypic characteristic was confirmed with the use of cellulose acetate electrophoresis at pH 8.5. All of the patients were receiving regular folate supplementation, and those <5 y of age were receiving standard oral prophylactic penicillin. The exclusion criteria were as follows: presence of other chronic diseases, blood transfusion in the previous 4 mo, hydroxyurea treatment, a history of overt stroke, or pregnancy. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, University of Khartoum, Sudan, and the Research Ethics Committee of Southampton & South West Hampshire, United Kingdom (REC reference number-05/Q1702/48). Self- or investigator-read and -explained written consent was obtained from the participants or their parents or guardians. The study was conducted in compliance with the established standard methodologic procedures and reported according to the revised Consolidated Standards of Reporting Trials statement.

Randomization and blinding

The subjects, after stratification by age and sex, were randomly assigned to receive coded and indistinguishable omega-3 or placebo capsules. Randomization was conducted by using a sequence of computer-generated random numbers at the Faculty of Life Sciences, London Metropolitan University (United Kingdom). The person who performed randomization had no knowledge about demographic, clinical, or laboratory characteristics of the patients, and the staff of the Sickle Cell Disease Referral Clinic, investigators, and participants were blinded until the biochemical and clinical outcome data were analyzed and the database unlocked.

Procedure

Subsequent to randomization, the patients were given, daily for 1 y, 1 (2–4-y-old; median weight: 13 kg), 2 (5–10; median weight: 25 kg), 3 (11–16; median weight: 37 kg), or 4 (17–24; median weight: 51 kg) omega-3 or placebo capsules. The

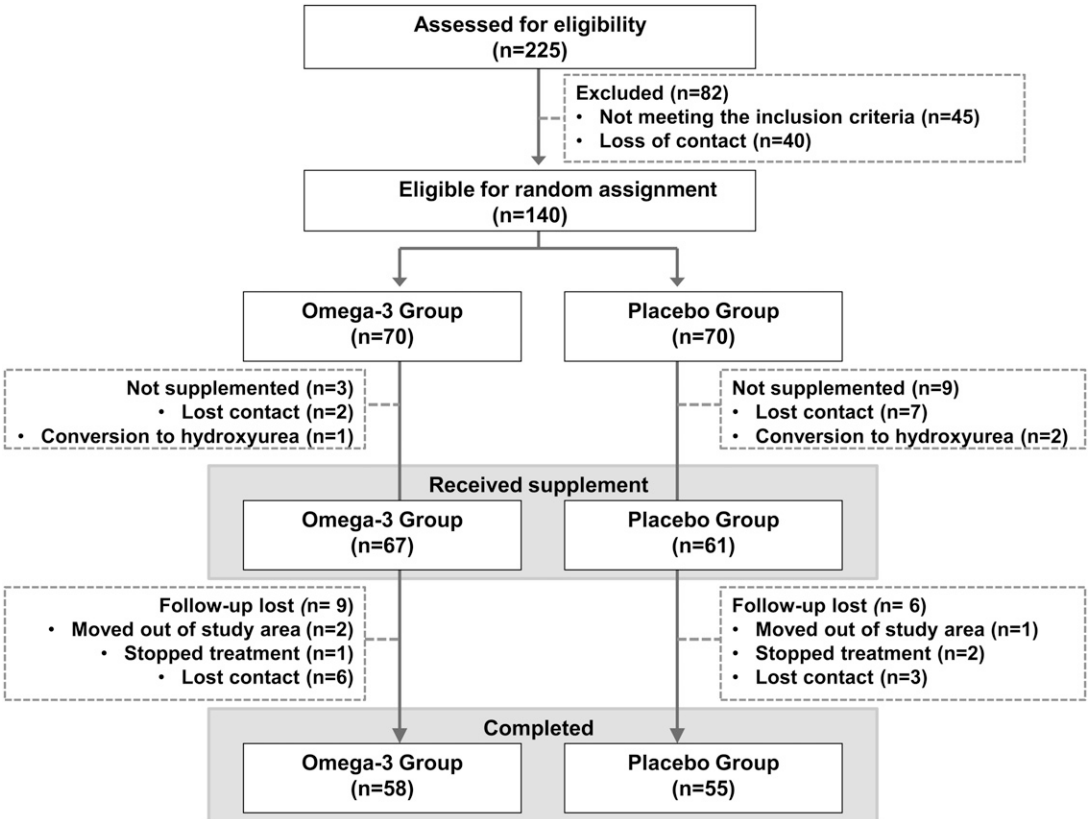

FIGURE 1. Consolidated Standards of Reporting Trials flowchart of patient enrollment, random assignment, and follow-up.

TABLE 1
Demographic and clinical characteristics of the patients[1]

	Omega-3 group	Placebo group
Number of patients (*n*)	70	70
Male	41	38
Female	29	32
Age (y)	8.1 ± 4.6[2]	7.8 ± 5.5
Weight (kg)	21.3 ± 10.5	21.8 ± 9.7
Height (cm)	117.5 ± 21.7	120.0 ± 22.6
Crisis-induced hospitalization (no./y)		
No admission	9.8	10.6
1–2	43.7	48.7
3–5	24.1	24.1
>5	22.4	16.7

[1] There was no significant difference between the 2 groups.
[2] Mean ± SD (all such values).

omega-3 capsule contained 277.8 mg DHA and 39.0 mg EPA, and the placebo capsule contained high oleic acid (41%) oil blend. The omega-3 fatty acid dose was calculated by multiplying the median weight of each age group by 25 mg/kg body wt. Vitamin E (1.5 mg/capsule) was added to both types of capsules to prevent peroxidation. Both types of capsules were carefully matched in appearance and flavor to prevent treatment unmasking. Enrollment identification number, sex, residence, ethnicity, weight, height, history of blood transfusion and stroke, number of sickle cell–related hospital admissions during the previous years, and sickle cell complication data were collected by using a validated structured questionnaire at baseline. A monthly self-assessment health diary was given to each patient or his or her guardian in which to record daily pain frequency and intensity, pain medication use, and hospitalizations. The name and telephone number of the medical doctor in charge was given to the patients and their guardians in case they required advice or care outside normal working hours. During each monthly follow-up, the self-recorded health diaries were reviewed, patients were examined thoroughly, and the data were obtained and entered into the database by the same physician. After 1 y of follow-up, 1203 health records were collected. Whole blood, ~10 mL, was obtained from the patients at recruitment and after 1 y of intervention for hematologic and biochemical analyses. The fatty acid composition of red blood cell membrane phosphoglycerides was assayed, and the choline and ethanolamine phospholipids are reported, as described in our previous publication (33).

The primary endpoint was the annualized rate of clinical vaso-occlusive crisis, defined as painful events that lead to hospitalization. Vaso-occlusive crisis was defined as a painful event characterized by musculoskeletal and/or visceral pain, which is usually associated with mild pyrexia and the passage of dark or red urine (34). The secondary endpoints were incidence of severe anemia (hemoglobin concentration <50 g/L), number of inpatient days due to clinical vaso-occlusive crisis, rate of blood transfusion, school attendance, mean hemoglobin concentration, and mean corpuscular volume. The number of inpatient days was used as an objective measure of the severity of clinical vaso-occlusive crisis. The design did not include active surveillance of harm because omega-3 fatty acids are found in commonly consumed food products, and previous human supplementation studies did not report adverse effects. Nevertheless, the participants were instructed to systematically record abnormal or undesirable reactions.

Statistical analysis

About 85% of the patients who attend the outpatient Sickle Cell Referral Clinic, Ibn-Aoaf Paediatric and Khartoum Teaching Hospitals, Khartoum (Sudan) develop ≥1 vaso-occlusive crises a year. It was assumed that this would be reduced to 60% through omega-3 fatty acid therapy. To detect a 25% difference in vaso-occlusive crisis between the omega-3 and placebo groups, with 80% power at a 5% significance level based on Fleiss with continuity correction, 56 patients would be required in each arm of the study. The total number of participating patients was increased to 140 to compensate for an anticipated 25% loss to follow-up.

The data are presented as means ± SDs, medians, and percentiles or medians and IQRs as appropriate. The vaso-occlusive crises were summarized on the basis of the annualized crisis rate by dividing the total number of crises experienced by the number of follow-up months and multiplying by 12. (A patient who experienced 3 crises and was followed up for 11 mo will have a crisis rate of 3.3/y.) The analyses were undertaken on an

TABLE 2
Complete blood count at baseline and after 1 y of the intervention[1]

	Omega-3 group (*n* = 67)		Placebo group (*n* = 61)		
	Baseline	1 y	Baseline	1 y	*P* value[2]
Hemoglobin (g/L)	67.1 ± 8.5	76.3 ± 8.5	67.5 ± 8.3	77.0 ± 10.2	<0.0001
Hematocrit (%)	21.1 ± 3.3	21.0 ± 2.3	22.3 ± 3.0	21.7 ± 3.6	>0.05
MCV (fL)	80.1 ± 5.6	82.7 ± 6.1	79.3 ± 6.6	80.6 ± 7.2	<0.0001
MCH (pg/L)	24.9 ± 2.1	30.4 ± 2.5	24.7 ± 2.4	29.4 ± 3.1	<0.0001
MCHC (g/dL)	30.9 ± 0.8	36.6 ± 1.4	31.2 ± 1.1	36.7 ± 1.3	<0.0001
TWBC ($\times 10^3/\mu$L)	15.6 ± 4.0	14.4 ± 3.3	14.5 ± 5.3	14.0 ± 5.1	<0.01
Platelet count ($\times 10^3/\mu$L)	516.8 ± 148.8	487.4 ± 114.7	496.8 ± 146.6	450.7 ± 165.1	0.02

[1] All values are means ± SDs. ANOVA and post hoc analysis were used to assess differences between and within the omega-3 and placebo groups and between the 2 time points when significance was indicated. There were no significant time-by-group interactions. MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; TWBC, total white blood cell count.
[2] Reflects the comparison of the combined group between baseline and after 1 y of the intervention.

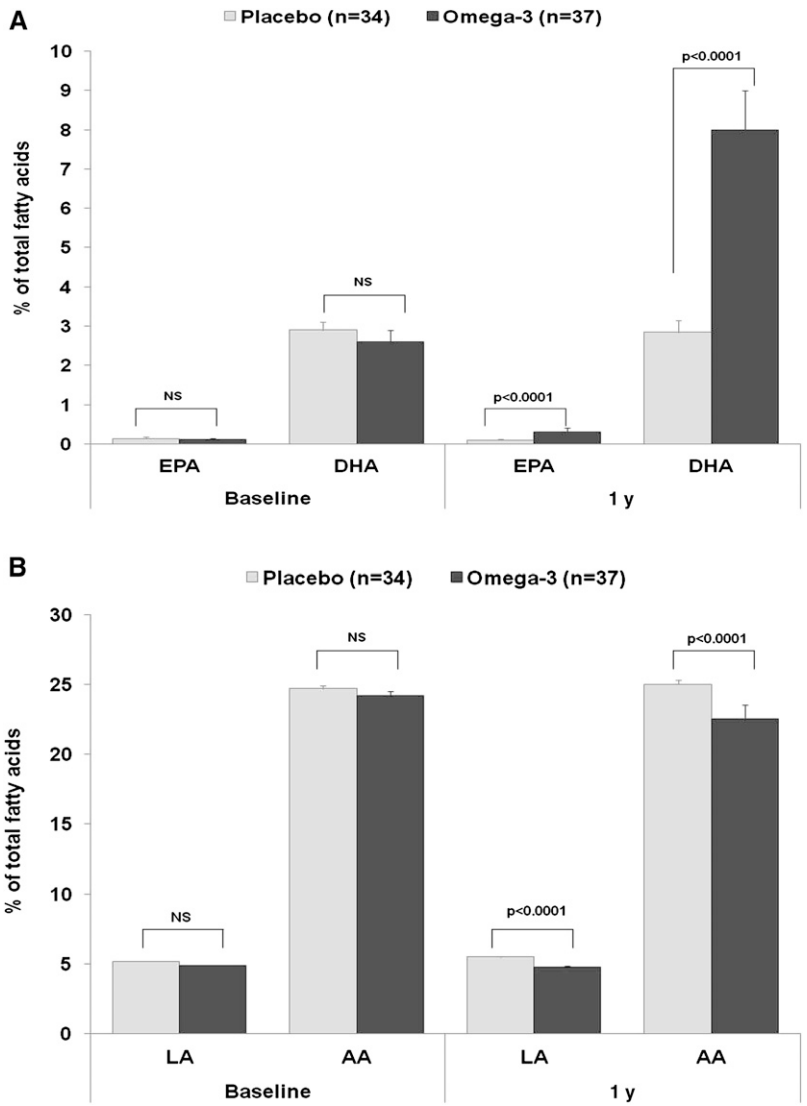

FIGURE 2. Mean (±SD) percentages of EPA, DHA, LA, and AA in red blood cell ethanolamine phosphoglyceride at baseline and after 1 y of follow-up. The 2 groups had comparable values of EPA, DHA, LA, and AA at baseline; however, after 1 y of intervention, EPA and DHA were higher and LA and AA were lower in the omega-3 group than in the placebo group. In the placebo group, percentages of EPA, DHA, LA, and AA were similar between baseline and after 1 y of intervention. In the omega-3 group, a significant increase in EPA and DHA values ($P < 0.0001$) and a reduction in AA values ($P < 0.0001$) were found after 1 y of intervention. The percentage of LA remained unaltered. Time-by-group interaction: LA ($P = 0.037$) and AA, EPA, and DHA ($P < 0.0001$). AA, arachidonic acid; LA, linoleic acid.

intention-to-treat basis by including all of the randomly assigned patients ($n = 140$). The treatment effects on clinical vaso-occlusive crisis, vaso-occlusive crisis, severe anemia, blood transfusion, and school attendance were tested after correction for the covariate effects of sex, age, and history of hospital admission due to vaso-occlusive crisis, and the duration of the follow-up was used as an offset. The Poisson regression or negative binominal model was fitted by using the Gamlss package in R software, depending on the dispersion of the data set. The equidispersed (equal variance and mean) clinical vaso-occlusive crisis data were analyzed by using the Poisson regression model and the overdispersed (variance greater than the mean) vaso-occlusive crisis data by using the negative binominal regression model. Multiple imputation was used to correct for missing data. The effect of the treatment on sequestration crisis, avascular necrosis, and stroke was assessed with Fisher's exact test. Statistical differences between the continuous variables were evaluated by ANOVA and by post hoc analysis when significance was indicated. The SPSS (version 17; Woking) and R (R Development Core Team, 2012) software programs were used for statistical analyses.

RESULTS

A total of 225 patients with HbSS were screened for eligibility, and 140 patients who fulfilled the inclusion criteria were randomly assigned to receive either omega-3 or placebo. Twelve of these patients dropped out and 128 were followed up and the data obtained. Three (2 in the placebo group and 1 in the omega-3 group) of the 12 who dropped out between randomization and intervention were converted to hydroxyurea, and the remaining 9 moved away from the area and could not be contacted. The

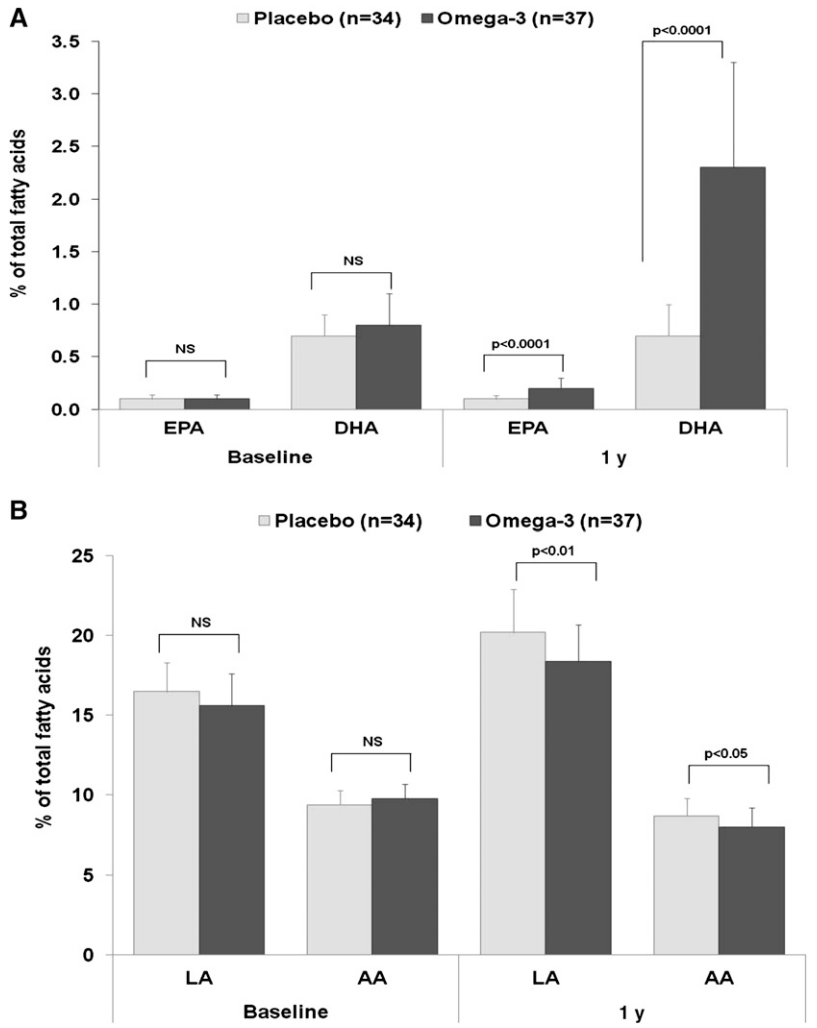

FIGURE 3. Mean (±SD) percentages of EPA, DHA, LA, and AA in red blood cell choline phosphoglyceride at baseline and after 1 y of follow-up. The 2 groups had comparable percentages of EPA, DHA, LA, and AA at baseline; however, after 1 y of intervention, EPA and DHA were higher and LA and AA were lower in the omega-3 group than in the placebo group. In the placebo group, the percentages of EPA and DHA were similar between baseline and after 1 y of intervention. However, a significant increase in LA ($P < 0.001$) and a reduction in AA ($P < 0.01$) were observed after 1 y of intervention. In the omega-3 group, significant increases were found in EPA and DHA ($P < 0.001$) and LA ($P < 0.0001$), whereas the AA percentage was significantly reduced ($P < 0.0001$) after 1 y of intervention. Time-by-group interaction: LA ($P > 0.05$), AA ($P < 0.001$), and EPA and DHA ($P < 0.0001$). AA, arachidonic acid; LA, linoleic acid.

Consolidated Standards of Reporting Trials flowchart of patient enrollment, randomization, and follow-up is shown in **Figure 1**. Seventeen patients (11 in the omega-3 group and 6 in the placebo group) had a follow up-period of <6 mo, and the median follow-up period was 10 mo.

At baseline, no difference was found in mean age, weight, height, and number of hospital admissions due to a sickle cell–induced crisis during the year before supplementation (**Table 1**); hematologic variables (**Table 2**); or percentage red blood cell membrane omega-3 fatty acids (EPA and DHA; **Figure 2**A and **Figure 3**A) and omega-6 (n−6; LA, and AA; Figures 2B and 3B) between the 2 groups. However, the omega-3 group had higher concentrations of EPA and DHA (Figures 2A and 3A, $P < 0.0001$) and lower concentrations of LA ($P < 0.01$) and AA ($P < 0.05$) (Figures 2B and 3B) in red blood cell choline and ethanolamine phosphoglyceride compared with their placebo counterparts after 12 mo of treatment.

The primary outcome, clinical vaso-occlusive crisis (median rate: 0 compared with 1; $P < 0.0001$), the number of hospitalization days due to sickle cell crisis and associated complications [median rate of 0 (IQR: 1) compared with median rate of 0 (IQR: 6); $P < 0.05$], and annualized vaso-occlusive crisis regardless of hospitalization (median rate: 2.7 compared with 4.6; $P < 0.01$) were lower in the omega-3 group than in the placebo group after 12 mo of intervention (**Table 3**, **Figure 4**). In the omega-3 group, the OR of having clinical vaso-occlusive crisis at least once during the study period was reduced to 0.21 (95% CI: 0.09, 0.47; $P < 0.001$). Similarly, the patients taking omega-3 had a lower incidence of severe anemia (3.0 compared with 16.4; $P < 0.05$) and blood transfusion rate (4.5 compared with 16.4; $P < 0.03$) (**Table 4**). No significant difference in percentage sequestration crisis (1.5 compared with 3.3), stroke (0 compared with 3.3), and avascular necrosis (1.5 compared with 3.3) was found between the omega-3 and placebo groups (Table 4).

TABLE 3
Annual rates of vaso-occlusive and clinical vaso-occlusive crises after 1 y of intervention[1]

	Omega-3 group (n = 70)	Placebo group (n = 70)
Vaso-occlusive crisis[2],*		
Minimum value	0	0
25th percentile	0.9	3.0
Median value	2.7	4.6
75th percentile	4.8	6.4
Maximum value	12.0	12.0
Clinical vaso-occlusive crisis[3],**		
Minimum value	0	0
25th percentile	0	0
Median value	0	1.0
75th percentile	0.9	2.4
Maximum value	4.0	6.0

[1] An intention-to-treat analysis using multiple imputation (30 data sets) to account for loss to follow-up was used to analyze the data. The overdispersed (variance greater than the mean) vaso-occlusive crisis data were analyzed with a negative binomial regression model and the equidispersed (equal variance and mean) clinical vaso-occlusive crisis data with a Poisson regression model. *,**Significant difference between omega-3 and placebo groups: *$P < 0.01$, **$P < 0.0001$.
[2] Defined as a painful crisis that may or may not have led to hospitalization.
[3] Defined as a painful crisis that led to hospital presentation.

No differences in hematologic variables were found between the omega-3 and placebo groups at baseline or after 1 y of intervention. Both the omega-3 and placebo groups had higher hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin (MCH), and MCH concentration at 12 mo compared with baseline values ($P < 0.001$) (Table 2).

The effect of omega-3 supplementation on school attendance was assessed in 95 patients (placebo, n = 46; omega-3, n = 49); 33 patients could not be tested because 31 were younger than school age and 2 were not in school. The median (IQR) numbers of absence days from school due to sickle cell–related illness were 0 (7.6) in the omega-3 group and 4.3 (21.1) in the placebo group. This difference was not statistically significant (**Table 5**). Omega-3 treatment reduced the OR of inability to attend school at least once during the study period due to sickle cell–related illness to 0.4 (95% CI: 0.2, 0.9; $P < 0.05$).

Four patients, 2 from each group, complained of dyspepsia, and 21 from the omega-3 group reported increased appetite during the initial period.

DISCUSSION

Supplementation of patients with HbSS with the omega-3 fatty acids DHA and EPA was effective at reducing the frequency and severity of vaso-occlusive episodes, severe anemia, and blood transfusion rate. These beneficial effects were reflected in noticeable improvements in health and related quality of life as evaluated by significant reductions in the number of inpatient hospital days and improvements in school absence due to sickle cell–related illness.

This study, to the best of our knowledge, is the first well-powered randomized, double-blind, placebo-controlled trial that investigated the therapeutic potential of omega-3 fatty acids for children and adults with HbSS. There is one published randomized study that reported a reduction in vaso-occlusive crises in patients with sickle cell disease supplemented with fish oil–containing omega-3 fatty acids. However, this study was based on a very small number of patients (n = 10, 5 in each arm), and only adults with frequent pain episodes were enrolled (31). These 3 limitations were controlled for in the current study.

Supplementation had no effect on stroke, sequestration crisis, and vascular necrosis. These findings were not surprising because the trial was not sufficiently powered to detect an effect on the aforementioned complications. Nevertheless, the study indicated general positive trends, which may need further exploration. The justification is perhaps stronger for stroke because the consumption of omega-3 fatty acids from fish is thought to protect against cerebral infarction (35).

Hemoglobin and MCH concentrations increased to the same extent in both groups after 1 y of treatment. Evidence indicates that vitamin E supplementation of patients with sickle cell disease increases hemoglobin concentration, percentage fetal hemoglobin, forearm blood flow, and cell resistance to lysis (36). Hence, the observed increase in hemoglobin and MCH, which appears to be unrelated to omega-3 fatty acid treatment, could be due to the effect of the vitamin E incorporated in the capsules and/or to improved medical care associated with monthly follow-up visits to Sickle Cell Referral Clinic.

In contrast with the earlier pilot studies (31, 32), high DHA and low EPA supplementation was used in the current investigation. They were used for the following reasons: *1*) the reduction of DHA is more pronounced than that of EPA in the blood cell membranes of HbSS patients (29, 30); *2*) EPA is a potent inhibitor of platelet aggregation (37), and a low level was used as a precaution against the risk of brain hemorrhage; and *3*) DHA has a greater influence on cell membrane deformability and fluidity than does EPA because of its higher unsaturation index (38). The mean percentages of DHA and EPA in the red blood cells of the Sudanese patients with sickle cell disease at baseline were lower than those in their Nigerian and British counterparts, who were studied previously by our group (39, 40). These differences are most likely a reflection of the omega-3 status of the Sudanese population, because the breast milk (41) and red blood cells of healthy children (33) from that country contain very low concentrations of these nutrients. After supplementation, nearly

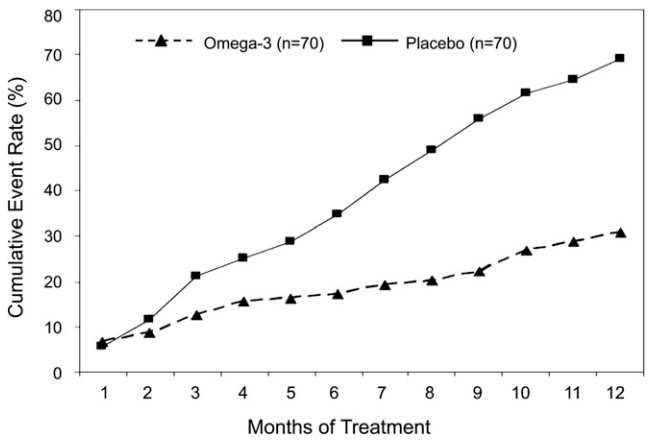

FIGURE 4. Cumulative event rate of clinical vaso-occlusive crises in the omega-3 and placebo groups.

TABLE 4
Sickle cell–associated complications in the omega-3 and placebo groups after 1 y of intervention[1]

	Omega-3 group (n = 70)		Placebo group (n = 70)		P value
	%	n	%	n	
Severe anemia	3.2	2	16.4	10	<0.05
Sequestration crisis	1.5	1	3.3	2	NS
Avascular necrosis	1.5	1	3.3	2	NS
Stroke	0	0	3.3	2	NS
Blood transfusion	4.5	3	16.4	10	<0.05

[1] The incidence of severe anemia and blood transfusion was analyzed by using a Poisson regression model. Statistical significance was assessed based on an intention-to-treat analysis with multiple imputation (30 data sets). The incidence of sequestration crisis, stroke, and avascular necrosis was analyzed by Fisher's exact test.

a 3-fold increase in red blood cell DHA and EPA and a concomitant reduction in the omega-6 fatty acid, AA, which is a precursor of the platelet aggregator thromboxane A2, was observed.

DHA, EPA, and their respective metabolites are known to exert a myriad of biochemical and biologic effects, directly and indirectly, including through competitive inhibition of actions of AA and its metabolites. However, the synergistic effects of decreased inflammation, blood cell aggregation, adhesion, and oxidative stress and of increased vasodilatation and blood flow (42, 43) may have played a critical role in the amelioration of vaso-occlusive and hemolytic crises in the patients.

The potential of omega-3 fatty acids to prevent neurologic and cognitive complications in children and adults and vaso-occlusive and severe anemia in children younger than 2 y were not investigated. Assessment of the severity of vaso-occlusive events was based solely on the number of inpatient hospital days; other objectives measures were not used. These limitations are potential lines of inquiry for future investigations. This randomized controlled trial, which was designed to evaluate the effects of omega-3 fatty acids on the primary and secondary endpoints and of sufficient duration to detect adverse events, showed that DHA and EPA supplementation prevents vaso-occlusive and hemolytic crises in patients with HbSS. If these findings are replicated in a large multicenter study, omega-3 fatty acids can be effective, safe, and affordable as a treatment for sickle cell anemia.

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The authors' responsibilities were as follows—KG: conceived the idea and initiated the study; KG, AAD, and MIE: designed the protocol; AAD and MIE: coordinated the implementation of the trial; AD, ZH, BA, and HHA: recruited and followed the patients and collected the clinical data; AAD: conducted the laboratory analysis; KG and AAD: analyzed and interpreted the data generated and wrote the manuscript; and MC: helped interpret the data and provided critical suggestions and comments. None of the authors disclosed a conflict of interest. The sponsors had no influence on the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to submit for publication.

TABLE 5
Absence from school after 1 y of intervention[1]

	Omega-3 group (n = 49)		Placebo group (n = 46)		
	Median	IQR	Median	IQR	P value
Absence from school per year (d)	0	7.6	4.3	21.1	NS

[1] Thirty-three patients (n = 31 younger than school age and 2 not attending school) were not included in the analysis. Zero-inflated Poisson regression was used to test for statistical significance.

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