European Review for Medical and Pharmacological Sciences 2022; 26: 5043-5052

Comparative effectiveness of adding Omega-3 or Vitamin D to standard therapy in preventing and treating episodes of painful crisis in pediatric sickle cell patients

S.M. ABDELHALIM[1,2], J.E. MURPHY[3], M.H. MEABED[4], A.A. ELBERRY[5,6], M.M. GAMALELDIN[7], M.S. SHAALAN[8], R.R.S. HUSSEIN[7,9]

[1]Department of Clinical Pharmacy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt, A Ph.D. supervision Committee with the Department of Pharmacy Practice & Science, R.K. Coit College of Pharmacy, University of Arizona, Tucson, AZ, USA
[2]Department of Pharmaceutical Sciences (Pharm-D program), Fakeeh College of Medical Sciences, Jeddah, Saudi Arabia
[3]Department of Pharmacy Practice & Science, R.K. Coit College of Pharmacy, University of Arizona, Tucson, AZ, USA
[4]Department of Pediatrics, [5]Department of Clinical Pharmacology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt
[6]Department of Pharmacy Practice, Pharmacy Program, Batterjee Medical College, Jeddah, Saudi Arabia
[7]Department of Clinical Pharmacy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt
[8]Department of Pharmaceutical Sciences (Pharm-D program), Fakeeh College of Medical Sciences, Jeddah, Saudi Arabia
[9]Department of Clinical Pharmacy, Faculty of Pharmacy, Modern University for Technology and Information, Cairo, Egypt

Abstract. – OBJECTIVE: Sickle Cell Anemia (SCA), also called the Sickle Cell Disease (SCD), is an inherited hematological disorder characterized by a syndrome of acute anemia and a painful crisis. The sickling hemoglobin, Hgb-S causes viscosity and inflammation of blood vessels. Eventually, the red blood cells get eliminated from the circulation process, which leads to hemolytic anemia. This study examined the comparative effectiveness of supplementation of Omega-3 or vitamin-D to standard therapy (hydroxyurea + Ibuprofen) used for prevention and treatment of pain crises in pediatric patients living with SCD.

PATIENTS AND METHODS: 165 patients participated in this randomized, double-blind, standard therapy-controlled, parallel-design trial. The patients were randomly divided into three groups, receiving three capsules of either 1,000 mg Omega-3 fish oil (400 mg EPA and 300 mg DHA) or 1.5 mL vitamin-D (2,800 IU/7 ml) daily for 10 months plus the standard therapy. Lactate dehydrogenase, high-density lipoprotein (HDL), low-density lipoprotein (LDL), hematocrit, reticulocyte count, and white-blood-cell count were determined at baseline (month zero) and end of the 10th month. The pain severity was measured using the visual analog scale method (VAS). Therefore, a 10-cm ruler with a VAS design was used to determine the patient pain intensity. The baseline time point was defined as the time spot before starting to deliver the experimental medication to the patients (month zero). At that time, the biodata of the patient on the frequency of pain episodes and the rest of the variables were collected, and the baseline data were one-year retrospective data.

RESULTS: Of 165 patients enrolled in the trial, 150 were included in the final analysis. At the end of the study, there was a significant increase in serum LDL and HDL in the Omega-3 group as compared with the control group (mean of 82 mg/dL *vs.* 57 mg/dL; $p < 0.01$ and mean of 47 mg/dL *vs.* 43 mg/dL; $p < 0.028$, respectively). Other laboratory parameters were significantly influenced. The number of painful crises and pain levels was significantly decreased in the Omega-3 group compared with the control group (mean of one-episode vs. mean of three episodes; $p = 0.01$, mean of three on pain scale vs. six on pain scale; $p = 0.018$).

Corresponding Author: Shaimaa M. Abdelhalim, MSc; e-mail: drshimaa.nashat@gmail.com
smabdelhalim@fcms.edu.sa

CONCLUSIONS: Results showed that Omega-3 was more effective than vitamin-D or standard treatment alone relative to pain crises and most of the other studied items. Vitamin-D was more effective than standard therapy alone. Clinicians should consider the addition of Omega-3 supplements to the standard therapy and a de-escalation dose plan for the hydroxyurea medication.

Key Words:
Omega-3 supplementation, Painful crisis, Sickle cell disease (SCD), Sickle cell anemia, Sickling, Hydroxyurea, Vaso-occlusive pain crises.

Introduction

Sickle Cell Anemia (SCA) is an inherited hematologic disease attributed to genetic transfer that has impacted the lives of many individuals worldwide. From a global perspective, about 20 to 25 million people are affected by SCD. Of those, 50% live in Africa and 100,000 individuals are affected in the United States[1]. There is a low incidence among the Egyptian population, except in rural areas like oases[2]. The carrier rate for SCD in Egypt has been reported as 9% to 20%[3]. The number of affected patients overall makes it important to understand and treat this condition effectively.

Patients with SCA experience sickling of their red blood cells (RBCs) due to hemoglobin-S[4], which leads to blocking of microcirculation. The common clinical feature present in this disease is pain caused by the distortion of red blood cells and the subsequent blocking of microcirculation. The painful crises caused by vaso-occlusion (VO) that are associated with SCA and the harmful effects associated with SCA are managed through both pharmacological (drugs and supplements) and non-pharmacological (bed rest, oxygen, and hydration) treatment.

One of the causes of SCA complications are deficiencies in certain vitamins (vitamins A, B6, C, D, E)[5]. It has been reported that vitamin D deficiency is linked to painful crises in SCA patients[6]. Lipid peroxidation, vascular adhesion, and low fatty acid composition in the RBC membrane is considered the cause of vascular inflammation that impacts the painful crises in SCA patients, making Omega-3 an option for treatment[7,8]. Vitamin D and Omega-3 supplementation have been reported to have a positive therapeutic impact on SCA patients[7-9], highlighting the need to investigate the impact of both Omega-3 and vitamin D in the painful crises of SCA patients.

SCA is treated using various medical interventions, such as blood transfusion, which decreases the amount of Hgb-S and reduces sickle cell erythropoiesis[10]. Blood transfusion is mostly preferred during the symptomatic stage of VO and also aids in the prevention of acute stroke[10]. Additional products and treatments available in the United States, but not all other countries, currently include L-glutamine, crizanlizumab, and voxelotor, in addition to allogeneic hematopoietic stem cell transplantation, which can cure SCD but has many risks. Gene therapy is being evaluated as a possible cure in severe SCD. The major medical intervention for SCA is hydroxyurea, whose main impact is to increase hemoglobin F (fetal hemoglobin) concentration. This reduces sickling and can reduce pain and pain episodes among the victims[11]. Folic acid is also commonly used to aid in the production of red blood cells. It has been reported that hydroxyurea therapy in SCD demonstrated a mild, dose-dependent leukopenia, nail/skin hyperpigmentation, neutropenia, and reticulocytopenia.

Omega-3 and vitamin D supplementation have been used in SCD to help with several of the adverse consequences of the disease. Omega-3 has impacts on the blood rheology and RBC aggregation and deformability[12,13], reduces the hemolytic effect on RBCs[14,15], promotes anti-inflammatory effects[8], and decreases leukocytosis[16,17].

Vitamin D deficiency is linked to the hemolysis process in SCD[18]. Vit-D acts as an antioxidant that reduces the hemolysis process, which can be measured by the hemolysis biomarkers reticulocyte percent and lactate dehydrogenase, which are reduced in response to the reduction of hemolysis[19]. As an anti-inflammatory, Vit-D promotes the anti-inflammatory cytokines, which is correlated to an increased anti-inflammatory activity[18,20,21].

The present study aimed at investigating and comparing the effectiveness of adding either Omega-3 or Vit-D to the standard therapy in reducing pain and painful crises among pediatric patients with SCD compared to standard therapy.

Patients and Methods

Study Design

The study was a parallel double-blind randomized controlled trial (1:1:1 allocation ratio), conducted from 2019-11-01 to 2020-11-01. Two

independent researchers were involved in the randomization process of the study. The first, who was blinded to the study procedures, conducted the random sampling and allocation using the random allocation software that was designed by Mahmood Saghaei[22]. The second implemented the randomized list using Microsoft Excel software version 2016. The randomized list included a unique ID number for each patient that was used to label each drug container delivered to patients by an independent nurse who was unaware of the study. Patients and clinicians involved in the study were not aware of the assigned groups. The blinding process was carried out by packing both experimental and standard treatment in identical drug containers, with no label except for that generated by the randomized list.

Study Population and Participants

The target population was pediatric patients attending the Beni-Suef and Giza governmental hospitals for their regular care at the pediatric hematology clinic. Patients aged 7-18 years old who presented with sickle cell anemia (HbSS mutation diagnosed with hemoglobin electrophoresis), and with the acute vaso-occlusive painful crisis were included. The patient condition was confirmed by clinical and laboratory investigations during regular care at the pediatric hematology clinic. Patients were also recruited from multiple hospitals in different Giza governmental hospitals to meet the minimum sample size estimation (150 patients). We also recruited five (5) additional patients in each group for counting future dropouts. The patients voluntarily participated in the trial through informed consent that was signed by their parents or legal guardians before randomization. Patients were placed in equal numbers into three study groups. Fifteen patients were excluded from the final statistical analysis because they did not follow up with the treating physician regularly (Figure 1).

The groups were: the standard therapy (hydroxyurea and folic acid +/- ibuprofen) control group (50 patients), the Omega-3 + standard therapy experimental group (50 patients), and the vitamin-D + standard therapy experimental group (50 patients). Patients excluded included who had had any other chronic diseases, hepatic diseases that might have altered the mechanism of action of the experimental drugs owing to the hepatic impairment, or renal disorders that could have caused pain owing to renal injuries, not due to the painful crises. This study was conducted according to the Helsinki declaration. It was approved by the Research Ethics Committee, Faculty of medicine Beni-Suef University, Beni-Suef, Egypt (FMB-SUREC/07072019/Sayed). The study was registered on clinicaltrials.gov, No. NCT04301336.

Study Outcomes

The impact of the experimental interventions and treatment duration on vaso-occlusive painful crises as measured by the nine clinical parameters described in Table I were investigated. The patients' clinical profiles and regular biochemical tests were assessed at baseline (month 0), and then every month until the end of the study (month 10)

Table I. Baseline[2] characteristics of the patients for the evaluated outcomes.

Variable	Control group (n-50)	Omega-3 group (n=50)	Vitamin D group (n=50)
Age (y)	10.5 ± 5.5	8.5 ± 4.0 1	12 ± 3.5
Gender (n)			
Male	35 (70%)	30 (60%)	25 (50%)
Female	15 (30%)	20 (40%)	25 (50%)
Number of painful episodes	3 ± 2	4 ± 2	5 ± 1
Pain level (VAS pain score)	6 ± 1	5.5 ± 1	5 ± 1.5
Hematocrit (%)	23 ± 6.5	23 ± 7.2	23 ± 4.2
HDL cholesterol (mg/dL)	34 ± 2	29 ± 3	31 ± 5
LDL cholesterol (mg/dL)	51 ± 13	56 ± 12	48 ± 15
White blood cell count (10^3/μL)	14.8 ± 3	15.5 ± 2.5	15 ± 1.4
Lactic acid dehydrogenase (U/L)	420 ± 25	329 ± 19	410 ± 30
Reticulocyte count (%)	6 ± 4	3 ± 0.4	5 ± 3
Hospitalization frequency	2 ± 3	3 ± 1	2 ± 2

Abbreviation: SD, standard deviation. [1]Mean ± SD (all such values), [2]baseline data of the prior 10-months of standard therapy alone which are reported at month zero.

to evaluate the effects of the interventions. The primary outcome measure was the number of painful crises. The secondary outcome measures were pain level (intensity), rate of hospitalization owing to painful attacks, laboratory and clinical parameters (LDL cholesterol, HDL cholesterol, lactate dehydrogenase (LDH), hematocrit, white blood cell (leukocytes) count, and reticulocyte count). A 10-cm line visual analog scale ruler was used to evaluate the patients' perceived level of pain. The pain level was scored on the ruler from 0 to 10, as 0 indicated "no pain" and 10 indicated "extreme pain". Venous blood (5 mL) was drawn into vacutainer tubes and transported to the hematology laboratory of hospitals where data were collected. Hematological parameters were measured including complete blood counts using a hematology analyzer. Lipid profile was measured using the automated procedures in the clinical chemistry laboratory of the hospital where patients' data were collected.

Experimental and Standard Therapy Interventions

All 165 patients in the study received standard therapy for painful vaso-occlusive crises. Standard therapy was hydroxyurea 20 mg/kg/day[11] for 8 to 10 months (maximum dose of 40 mg/kg/day) and folic acid 0.5 to 1 mg daily[23] for 3 to 4 weeks until definite hematologic response. All patients received ibuprofen for pain relief with an initial dose of 5 mg/kg/dose[24] followed by 1 to 3 additional doses as needed. Blood transfusions were used for patients in all groups when deemed necessary by treating clinicians. The two experimental therapies added to standard therapy were: Omega-3 supplementation [300-400 mg eicosapentaenoic acid (EPA) and 200-300 mg of docosahexaenoic acid (DHA)] per day[19-21,25] for 10 consecutive months or vitamin-D 1,500-3,500 IU per day for 10 consecutive months[26,27]. The adherence to study treatment was assessed every month at each follow-up visit by counting up the used treatment bottles received from the patients on each visit.

Statistical Analysis

The Statistical Package for the Social Sciences 25 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Kruskal-Wallis' test was used to analyze the difference in means among groups for the primary outcome (number of painful episodes). The normality of secondary outcomes was checked and approved by the Shapiro-Wilk test. Two-way ANOVA and post-hoc LSD tests were used to analyze the main effects and the interaction effects among the groups for the secondary outcomes. The assumptions for two-way ANOVA were checked. The significance level was maintained at $p<0.05$. Data were summarized as means, standard deviations (SD). The mean values for the primary outcome were separately measured from the secondary outcome in all groups. The differences of mean values (between treatment groups and control group) for the secondary outcomes were analyzed using two-way ANOVA analysis and post-hoc analysis. Secondary outcomes analyses and primary outcome analyses were conducted, separately from each other. The analysis of secondary outcomes was considered a sub-trial from this present trial. Power analysis with G-Power software (version 3.1) was used to determine the sample size. The sample size was set for all primary and secondary outcomes. The minimum sample size estimation for each group was 50 patients to detect a difference of 50% reduction in means value of the number of pain crises with a pooled standard deviation of 26, obtained from previous studies[28-30]. The calculated sample size was 165 patients, considering the dropouts, matched into two experimental interventions of which 55 patients in the vitamin-D group, 55 patients in the Omega-3 group, and 50 patients in the standard control group. The study had 80% of power with an 0.05 alpha level and a medium-size effect (partial $\eta^2 = 0.06$).

Results

Data were collected on 165 participants who met the inclusion criteria. Finally, 50 (mean age 8.5 ± 4, 60% male) were in the Omega-3 group, 50 (mean age 10.5 ± 3.5, 50% male) in the vitamin D group, and 50 (mean age 11 ± 5.5, 70% male) in the control group were analyzed. The flow chart of study is shown in Figure 1. Tables I and II show the primary results of the study, including the baseline characteristics of participants for the evaluated outcomes (Table I). Analysis of variance between the three groups investigating the main effects and the interaction effects (Table II, Figure 2, Figure 3) showed that Omega-3 supplementation to the standard therapy was more significant than vitamin-D and control groups. Omega-3 supplementation significantly reduced the number of painful crises (p=0.040), pain severity (p=0.044), and hospitalization frequency

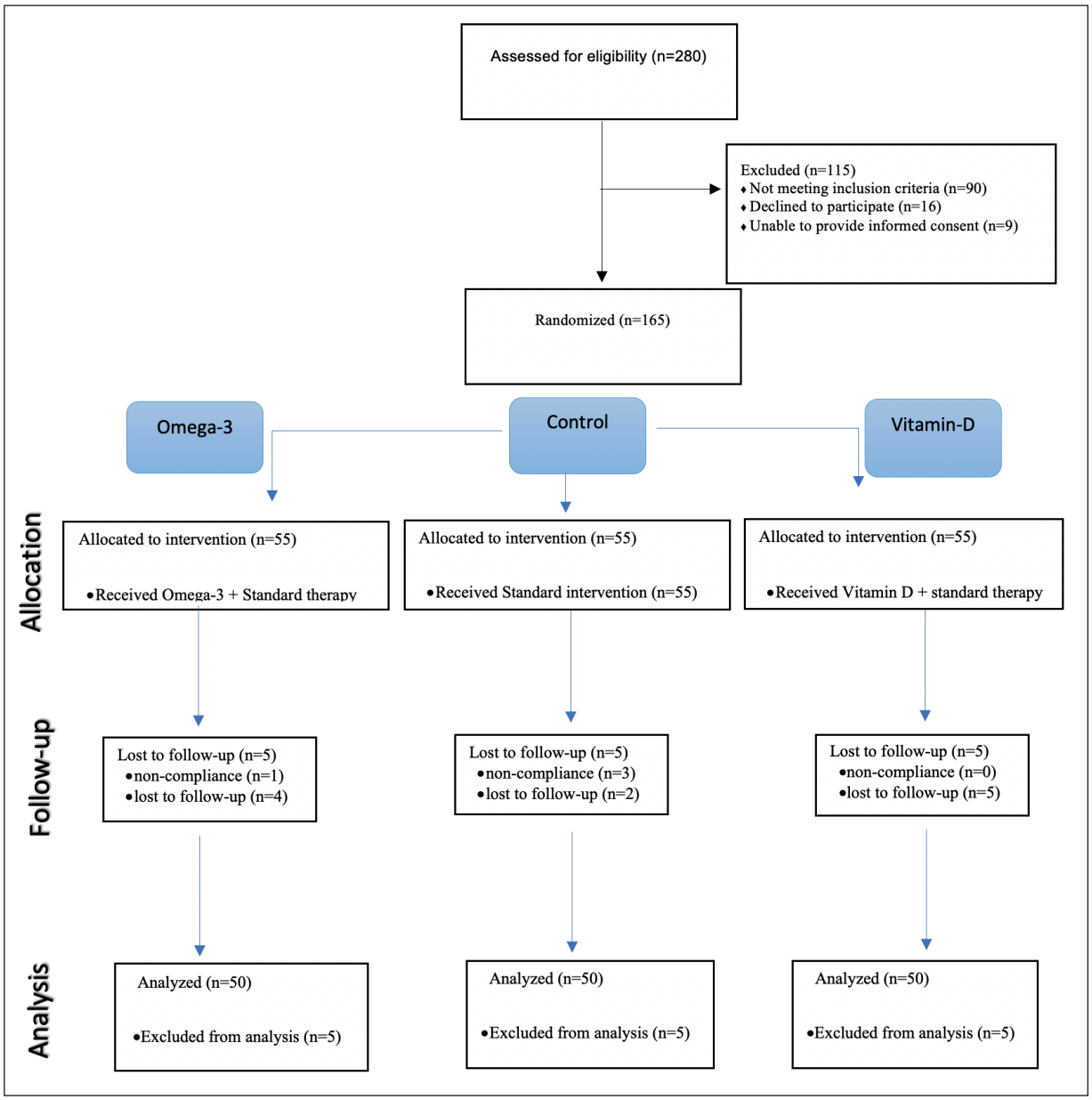

Figure 1. The follow chart of the study.

(p=0.038). In line with this, Omega-3 supplementation increased the LDL-cholesterol (p=0.030) and HDL cholesterol (p=0.020) significantly compared to vitamin-D and standard therapy. Both are linked with lipid peroxidation and sickle cell lipid composition. Omega-3 supplementation reduced leukocytosis significantly (p=0.048), which is linked with the intravascular inflammation of the painful crises. Similarly, the analysis showed that vitamin-D supplementation to standard therapy was significantly more effective than standard therapy. Vitamin-D supplementation significantly reduced the lactate dehydrogenase level (p=0.030) and reticulocyte count (p=0.045), which are linked to vascular inflammation and hemolysis process in sickle cells. All results are summarized in Table II.

Discussion

Serum level of vitamin-D are correlated to intravascular hemolysis in children with SCD. Thus, it may play an important role in the man-

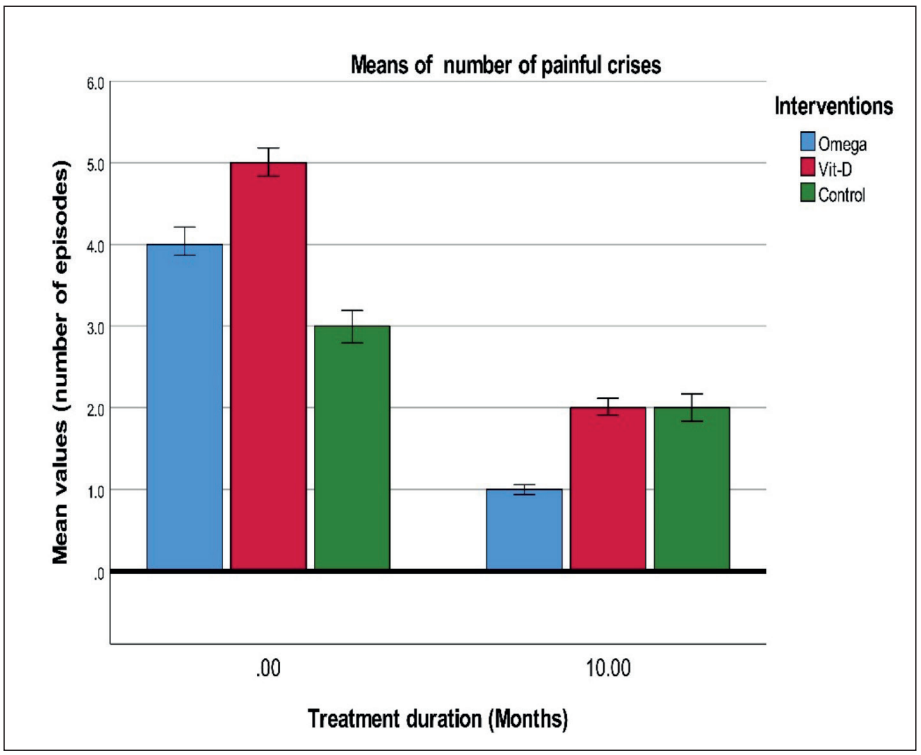

Figure 2. The effect of the experimental interventions on the number of painful episodes after ten months of treatments.

agement of painful crises[18]. In addition, previous studies[21,31] found a relationship between serum level of vitamin-D and inflammatory cytokines and hospitalization for pain crises in pediatric SCD. Recent studies investigated and confirmed the anti-inflammatory and antioxidant effects of vitamin-D. None of those studies concluded on the effects of vitamin-D on the painful crises of pediatric patients[32]. Omega-3 was found to have a potential effect on patients with SCD, even if there is no known mechanism for those effects on the painful crises in pediatric SCD[7]. Some clinical studies[38,39] investigated the antiaggregatory, antiadhesive, and anti-inflammatory function of Omega-3 supplements on SCD. However, those studies did not link those effects with the management of painful crises[12]. In addition, previous clinical studies[33] concluded that Omega-3 supplements had antioxidant effects on health and diseased individuals. Nevertheless, no evidence did confirm its effects on the management of vaso-occlusive pain in SCD patients. As evident, this present study investigated the effects of vitamin-D and Omega-3 on the management of painful crises in children patients.

Table II. Mean changes for the evaluated outcomes after ten months of treatment compared to baseline values (before treatment).

Variable	Control group Mean ± SD	Omega-3 group Mean ± SD	Vitamin D group Mean ± SD
Number of painful episodes	2±4.53	1±3.03$^{*, A}$	2±4.04$^{*, A}$
Pain level (VAS pain score)	6±9.071	3±7.010*	4±8.081$^{*, C}$
HDL cholesterol (mg/dL)	41±0.638	52±0.72*	46±.073$^{*, C}$
LDL cholesterol (mg/dL)	57±1.43	82±1.00*	68±1.20$^{*, C}$
White blood cell count (10^3/μL)	15±0.512	13.1±0.075$^{*, B}$	15±2.2
Lactic acid dehydrogenase (U/L)	331±24.41	328±22	297±32.19$^{*, C}$
Reticulocyte count (%)	3±.072	2.9±0.3	2±0.050$^{*, C}$
Hospitalization frequency	3±4.430	1±3.030*	2±4.040$^{*, C}$

*Significantly different from control group value at $p < 0.05$, ^{A}Kruskal-Willis' test, ^{B}Post-hoc comparison between mega-3 and control, ^{C}Post-hoc comparison between Vitamin-D and control.

Our findings indicated that adding Omega-3 supplementation (dose of 400 mg EPA and 300 mg DHA daily for 10 months) to the standard therapy had a significant therapeutic effect on the painful crises of SCD patients. The antioxidant effect of Omega-3 and vitamin-D offsets lipid peroxidation[34-37]. As a result, they increase the HDL and LDL levels significantly compared to standard therapy (hydroxyurea+ folic acid+ Ibuprofen) in SCD patients who demonstrated hypocholesteremia. Omega-3 supplementation and vitamin-D supplementation revert hypocholesteremia that has been correlated to the hemolysis effect on RBC[14,15], and the vascular inflammation of sickled erythrocytes[8]. Previous research studies[8] have found that hypocholesteremia has promoted lipid oxidation that is correlated with HbS level in a pain crisis and enhanced the proinflammatory factor in a pain crisis. Omega-3 supplementation in SCD enhances the lipid composition and stability of the sickled RBC membrane[32]. It has been shown to impact the blood rheology and aggregation of RBC in SCD patients[12,13]. In this study, the number of painful crises (Figure 2) and the level of pain (Figure 3) are significantly reduced with Omega-3 compared to vitamin-D and control groups. Vitamin-D significantly reduces the painful crises and the pain level compared to the control group.

Omega-3 supplementation significantly reduced the elevated white blood cells (leukocytosis) in the patients compared to the control group. Leukocytosis have been correlated to vascular inflammation and vascular adhesion during the pain crisis[16,17].

For the vitamin-D supplementation group, a 2,500 IU daily dose of vitamin-D for ten months has significantly affected the hemolysis biomarkers (reducing LDH and reticulocyte count) that reflect the vascular inflammation in the patients compared to the control group. LDH and reticulocyte levels have been linked to the hemolysis process, which results in vascular inflammation[19]. Vitamin-D deficiency has been correlated to the hemolysis process in SCD patients[18]. Results have indicated that the experimental interventions have demonstrated a cumulative response in reducing the painful crisis. Better outcomes were accomplished over a longer treatment duration and may continue longer than the studied 10 months. The blinding strategies undertaken in the study met the purpose of masking the experimental interventions. Although it would not completely mask the interventions as some patients were assigned to oral drops dosage form and

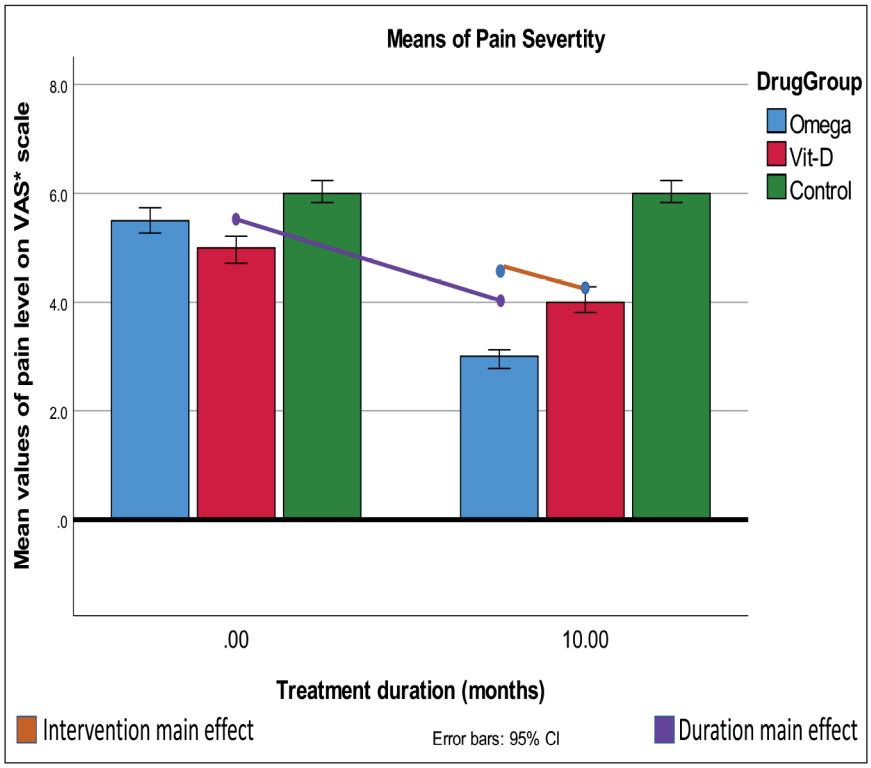

Figure 3. The effect of the experimental interventions on the pain level after ten months of treatments.

others assigned to the tablet dosage form, all were packed in identical containers masking the drug label from the participants, caring nurse, and the treating physician.

Conclusions

The research compared the effectiveness of the standard therapy of hydroxyurea and folic acid to the supplementation of Omega-3 or vitamin-D to standard therapy in the treatment of painful crises and a variety of associated laboratory parameters among patients living with SCA. Key findings from the study were that omega-3 was generally more effective than the other regimens. The effect of vitamin-D supplementation was lower, though often better than hydroxyurea/folic acid alone, making Omega-3 the best oral regimen to be added to standard therapy in suppressing the number and pain level of the painful crisis episodes among patients experiencing SCA.

Though there might be value in doing so, it is unknown whether combining both Omega-3 and vitamin-D with standard therapy might provide even better outcomes than the addition of Omega-3 alone to standard therapy. Omega-3 and vitamin-D are relatively inexpensive supplements, so their addition to standard therapy may be possible for many patients. Research on whether treating patients with the relatively inexpensive supplements might allow for reduction of doses of hydroxyurea and may be warranted to reduce side effects of the standard therapies.

Acknowledgments

This study is a part of Ph.D. dissertation at the Department of Clinical Pharmacy, Faculty of Pharmacy, Beni-Suef university, Egypt, with joint supervision committee at the Department of Pharmacy Practice & Science, R. K. Coit College of Pharmacy, University of Arizona, Arizona, USA. The authors would like to thank all the physicians and healthcare providers in Giza governmental hospitals who helped the principal investigator conducting the study. We would like to thank Dr. Ahmed Fatthi, a pediatric specialist at Beni-Suef insurance hospital who helped in collecting and supervising patients' data.

Authors' Contributions

The corresponding author thanks Prof. John E. Murphy and Prof. Mohamed Hussein Meabed for their contribution to the conception and design of the study, Dr. Ahmed A. Elberry for his contribution to the revision of the study, Dr. Raghda R.S. Hussein for his contribution to drafting and designing the study, Mr. Mohamed M. Gamaleldin for their contribution to acquisition and analysis of data, Mrs. M.S. Shaalan for her contribution to data interpretation.

Ethics Approval

The present study was conducted based on the Helsinki guidelines. This study was approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University, Egypt with a registration number of FMBSUREC/07072019/Sayed. The study has been registered on clinicaltrials.gov under the code: NCT04301336.

Informed Consent

All participants provided a signed informed consent.

Data Availability

The data that support the findings of this study are available on request from the corresponding author (Shaimaa M. Abdelhalim). Data are not publicly available due to their containing information that could compromise the privacy of research participants..

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Funding

The study received no funds.

ORCID ID

S.M. Abdelhalim: 0000-0001-5372-5337
A.A. Elberry: 0000-0002-0073-3066
M.M. Gamaleldin: 0000-0002-5188-637X
M.S. Shaalan: 0000-0001-8739-5030
R.R.S. Hussein: 0000-0002-0503-685X

References

1) Hood AM, Quinn CT, King CD, Shook LM, Peugh JL, Crosby LE. Vitamin d supplementation and pain-related emergency department visits in children with sickle cell disease. Complement Ther Med 2020; 49: 102342.

2) El-Beshlawy A, Youssry I. Prevention of hemoglobinopathies in egypt. Hemoglobin 2009; 33: 1-2.
3) Abou-Elew HH, Youssry I, Hefny S, Hashem RH, Fouad N, Zayed RA. Beta(s) globin gene haplotype and the stroke risk among egyptian children with sickle cell disease. Hematology 2018; 23: 362-367.
4) Russo G, De Franceschi L, Colombatti R, Rigano P, Perrotta S, Voi V, Palazzi G, Fidone C, Quota A, Graziadei G, Pietrangelo A, Pinto V, Ruffo GB, Sorrentino F, Venturelli D, Casale M, Ferrara F, Sainati L, Cappellini MD, Piga A, Maggio A, Forni GL. Current challenges in the management of patients with sickle cell disease - a report of the italian experience. Orphanet J Rare Dis 2019; 14: 120.
5) Dekker LH, Fijnvandraat K, Brabin BJ, van Hensbroek MB. Micronutrients and sickle cell disease, effects on growth, infection and vaso-occlusive crisis: A systematic review. Pediatr Blood Cancer 2012; 59: 211-215.
6) Osunkwo I, Hodgman EI, Cherry K, Dampier C, Eckman J, Ziegler TR, Ofori-Acquah S, Tangpricha V. Vitamin d deficiency and chronic pain in sickle cell disease. Br J Haematol 2011; 153: 538-540.
7) Daak A, Rabinowicz A, Ghebremeskel K. Omega-3 fatty acids are a potential therapy for patients with sickle cell disease. Nat Rev Dis Primers 2018; 4: 15.
8) Daak AA, Lopez-Toledano MA, Heeney MM. Biochemical and therapeutic effects of omega-3 fatty acids in sickle cell disease. Complement Ther Med 2020; 52: 102482.
9) Soe HHK, Abas AB, Than NN, Ni H, Singh J, Said A, Osunkwo I. Vitamin d supplementation for sickle cell disease. Cochrane Database Syst Rev 2020; 5: CD010858.
10) Howard J. Sickle cell disease: When and how to transfuse. Hematology Am Soc Hematol Educ Program 2016; 2016: 625-631.
11) Tshilolo L, Tomlinson G, Williams TN, Santos B, Olupot-Olupot P, Lane A, Aygun B, Stuber SE, Latham TS, McGann PT, Ware RE, Investigators R. Hydroxyurea for children with sickle cell anemia in sub-saharan africa. N Engl J Med 2019; 380: 121-131.
12) Daak AA, Ghebremeskel K, Hassan Z, Attallah B, Azan HH, Elbashir MI, Crawford M. Effect of omega-3 (n-3) fatty acid supplementation in patients with sickle cell anemia: Randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 2013; 97: 37-44.
13) Chang CH, Tseng PT, Chen NY, Lin PC, Lin PY, Chang JPC, Kuo FY, Lin J, Wu MC, Su KP. Safety and tolerability of prescription omega-3 fatty acids: A systematic review and meta-analysis of randomized controlled trials. Prostaglandins Leukot Essent Fatty Acids 2018; 129: 1-12.
14) Yalcinkaya A, Unal S, Oztas Y. Altered hdl particle in sickle cell disease: Decreased cholesterol content is associated with hemolysis, whereas decreased apolipoprotein a1 is linked to inflammation. Lipids Health Dis 2019; 18: 225.
15) Omar ZA, Montser BA, Farahat MAR. Effect of high-dose omega 3 on lipid profile and inflammatory markers in chronic hemodialysis children. Saudi J Kidney Dis Transpl 2019; 30: 634-639.
16) Gutierrez S, Svahn SL, Johansson ME. Effects of omega-3 fatty acids on immune cells. Int J Mol Sci 2019; 20: 5028.
17) Gultekin G, Sahin H, Inanc N, Uyanik F, Ok E. Impact of omega-3 and omega-9 fatty acids enriched total parenteral nutrition on blood chemistry and inflammatory markers in septic patients. Pak J Med Sci 2014; 30: 299-304.
18) Adegoke SA, Braga JAP, Adekile AD, Figueiredo MS. The association of serum 25-hydroxyvitamin d with biomarkers of hemolysis in pediatric patients with sickle cell disease. J Pediatr Hematol Oncol 2018; 40: 159-162.
19) Delesderrier E, Curioni C, Omena J, Macedo CR, Cople-Rodrigues C, Citelli M. Antioxidant nutrients and hemolysis in sickle cell disease. Clin Chim Acta 2020; 510: 381-390.
20) Elsayed MAO, Ibrahim GAA, Mohamed EMO, Osman AAA, Noureddin AA, Alawad MOA, Mohamedali AOO, Awadalla HFM, MohamedElmugadam FAA, Mohamed SOO. A meta-analysis on vitamin d deficiency in patients with sickle cell disease. Dr Sulaiman Al Habib Medical Journal 2020; 2: 95-100.
21) Adegoke SA, Smith OS, Adekile AD, Figueiredo MS. Relationship between serum 25-hydroxyvitamin d and inflammatory cytokines in paediatric sickle cell disease. Cytokine 2017; 96: 87-93.
22) Saghaei M. Random allocation software for parallel group randomized trials. BMC Med Res Methodol 2004; 4: 26.
23) Verhovsek M, Simpson S, Segbefia C, Pendergrast J, Amid A, Harris-Janz S, Kaufman M, Cheong M, Odame I. Consensus Statement on the Care of Patients with Sickle Cell Disease in Canada. Canadian Haemoglobinopathy Association, 2015.
24) Oshikoya KA, Edun B, Oreagba IA. Acute pain management in children with sickle cell anaemia during emergency admission to a teaching hospital in lagos, nigeria. SAJCH 2015; 9: 119-123.
25) Daak AA, Elderdery AY, Elbashir LM, Mariniello K, Mills J, Scarlett G, Elbashir MI, Ghebremeskel K. Omega 3 (n-3) fatty acids down-regulate nuclear factor-kappa b (nf-kappab) gene and blood cell adhesion molecule expression in patients with homozygous sickle cell disease. Blood Cells Mol Dis 2015; 55: 48-55.
26) Mazzoleni S, Magni G, Toderini D. Effect of vitamin d3 seasonal supplementation with 1500 iu/day in north italian children (dinos study). Ital J Pediatr 2019; 45: 18.
27) Osunkwo I, Ziegler TR, Alvarez J, McCracken C, Cherry K, Osunkwo CE, Ofori-Acquah SF, Ghosh S, Ogunbobode A, Rhodes J, Eckman JR, Damp-

ier C, Tangpricha V. High dose vitamin d therapy for chronic pain in children and adolescents with sickle cell disease: Results of a randomized double blind pilot study. Br J Haematol 2012; 159: 211-215.

28) Ballas SK, Bauserman RL, McCarthy WF, Castro OL, Smith WR, Waclawiw MA. Hydroxyurea and acute painful crises in sickle cell anemia: Effects on hospital length of stay and opioid utilization during hospitalization, outpatient acute care contacts, and at home. J Pain Symptom Manage 2010; 40: 870-882.

29) Bartolucci P, El Murr T, Roudot-Thoraval F, Habibi A, Santin A, Renaud B, Noel V, Michel M, Bachir D, Galacteros F, Godeau B. A randomized, controlled clinical trial of ketoprofen for sickle-cell disease vaso-occlusive crises in adults. Blood 2009; 114: 3742-3747.

30) Ebrahimi M, Ghayour-Mobarhan M, Rezaiean S, Hoseini M, Parizade SM, Farhoudi F, Hosseininezhad SJ, Tavallaei S, Vejdani A, Azimi-Nezhad M, Shakeri MT, Rad MA, Mobarra N, Kazemi-Bajestani SM, Ferns GA. Omega-3 fatty acid supplements improve the cardiovascular risk profile of subjects with metabolic syndrome, including markers of inflammation and auto-immunity. Acta Cardiol 2009; 64: 321-327.

31) Brown B, Long K, Agdere L, Kulpa J, Zarzoso-Fernandez S, Choudhary D, Sundarum R. The association between vitamin d deficiency and hospitalization outcomes in pediatric patients with sickle cell disease. Blood Cells Mol Dis 2020; 82: 102415-102415.

32) Osunkwo I, Manwani D, Kanter J. Current and novel therapies for the prevention of vaso-occlusive crisis in sickle cell disease. Ther Adv Hematol 2020; 11: 2040620720955000.

33) Tc K, Wnb D, Marlyne M, Ac P, Kansci G, Fokou E. Antisickling and antioxidant properties of omega-3 fatty acids epa / dha. Nutri Food Sci Int J 2019; 9: 1-6.

34) Meital LT, Windsor MT, Perissiou M, Schulze K, Magee R, Kuballa A, Golledge J, Bailey TG, Askew CD, Russell FD. Omega-3 fatty acids decrease oxidative stress and inflammation in macrophages from patients with small abdominal aortic aneurysm. Sci Rep 2019; 9: 12978.

35) Oppedisano F, Macri R, Gliozzi M, Musolino V, Carresi C, Maiuolo J, Bosco F, Nucera S, Caterina Zito M, Guarnieri L, Scarano F, Nicita C, Coppoletta AR, Ruga S, Scicchitano M, Mollace R, Palma E, Mollace V. The anti-inflammatory and antioxidant properties of n-3 pufas: Their role in cardiovascular protection. Biomedicines 2020; 8: 306.

36) Hajianfar H, Paknahad Z, Bahonar A. The effect of omega-3 supplements on antioxidant capacity in patients with type 2 diabetes. Int J Prev Med 2013; 4: S234-S238.

37) Kim HA, Perrelli A, Ragni A, Retta F, De Silva TM, Sobey CG, Retta SF. Vitamin d deficiency and the risk of cerebrovascular disease. Antioxidants (Basel) 2020; 9: 327.

38) Daak AA, Lopez-Toledano MA, Heeney MM. Biochemical and therapeutic effects of omega-3 fatty acids in sickle cell disease. Complement Ther Med 2020; 52: 102482.

39) Daak A, Rabinowicz A, Ghebremeskel K. Omega-3 fatty acids are a potential therapy for patients with sickle cell disease. Nat Rev Dis Primers 2018; 4: 15.