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Assessment the Effect of Omega-3 Fatty Acid Supplementation in Sudanese patients with Sickle Cell Anemia; Khartoum, Sudan

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Abstract

Background: *Sickle cell disease (SCD) is a group of 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.*
Objectives: *This study aimed to assess the effect of omega-3 supplementation in patients with sickle cell anemia.*
Subjects and methods: *Seventy-eight SCD patients, who were undergoing regular follow-up at the outpatients SCD clinic in Ibn-Aoaf Pediatrics and Khartoum hospitals, were the main study group and 35 individuals were selected as control group. Patients recruited randomly were assigned to receive omega-3 fatty acid supplementation for at least two years. 5 ml of venous blood was collected from every individual into EDTA test tubes and used to determine complete blood count (CBC), electrophoresis and reticulocyte count.*
Results: *A significant difference in the Hb (g/dl) mean between the omega-3 group and the group free of omega-3 (P. value < 0.0001) was recorded. Also in total red blood cells count X 103C/M l, hematocrit and mean cell hemoglobin pg (P.value < 0.05) .No difference in levels between the study groups at mean cell volume / f l and mean cell hemoglobin concentration % (P. value < 0.05). Retics count and reticulocyte production index significant decrease in omega-3 group compared to control group (P.value < 0.0001).*
Conclusions: *These findings suggest that omega-3 fatty acids can be an effective, safe, and affordable therapy for sickle cell anemia.*

Keywords: *Sickle cell anemia, Omega-3 Fatty Acid, Khartoum, Sudan.*

Introduction

SCD 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), also known as sickle cell anemia, is the major and severest form of the disease2. In sub-Saharan Africa, the prevalence of the sickle cell trait ranges between 5% and 40% of the population, and >230,000 (0.74% of total birth) infants are born with sickle cell anemia every year3. Vaso-occlusive crisis is the main clinical manifestation, accompanied by hemolysis and is the main cause of hospitalization and requiring blood transfusion to avoid organ damage, and death. More than 10% of patients with sickle cell anemia develop overt stroke, and 22% show evidence of silent cerebral infarction. In Africa, the life expectancy of patients with sickle cell disease is less than 20 years, and those less than 5 years of age are at the highest risk of death4. It has been reported that supplementation with fish oil containing the omega-3 (n−3) fatty acids Eicosapentaenoic acid and Docosahexaenoic acid (EPA and DHA) reduces the frequency of pain episodes requiring hospital presentation and the number of sickle cell crises4.

In a previous retrospective study, it was observed that the greater the amounts of the omega-3 fatty acids (EPA) and (DHA) in the blood, the lesser the number of complications of sickle cell disease (SCD) and the higher the steady-state hemoglobin level.

Sickle Cell Disease: Nutritional Considerations

Patients with sickle cell disease have an increased need for calories and micronutrients (e.g., vitamins and minerals). A diet emphasizing fruits, vegetables, whole grains, and legumes will provide a greater proportion of essential nutrients than a typical Western diet, and appropriate supplementation (one to three times the recommended intakes for most essential nutrients) can prevent deficiency.

Omega-3 fatty acid supplements

Supplementation with omega-3 fatty acids can improve the membranes of red blood cells and may decrease flare-ups of the disease. A small preliminary study indicated that omega-3 fatty acid supplementation with fish oil reduced the number of painful episodes requiring hospitalization. However, this finding has not yet been confirmed in controlled trials.6

Omega-3 fatty acids

Although omega-3 fatty acids have been known as essential to normal growth and health since the 1930s[7], awareness of their health benefits has dramatically increased since the 1990s.[7]

Omega 3 fatty acids are fats commonly found in marine and plant oils. They are polyunsaturated fatty acids with a double bond (C=C) starting after the third carbon atom from the end of the carbon chain. The fatty acids have two ends:- the acid (COOH) end and the methyl (CH_3) end. The location of the first double bond is counted from the methyl end, which is also known as the omega (ω) end or the n end. The potential health benefits of n-3 fatty acids supplementation are controversial. They are considered essential fatty acids, meaning that they cannot be synthesized by the human body but are vital for normal metabolism. Though mammals cannot synthesize n−3 fatty acids, they have a limited ability to form the long-chain n−3 fatty acids including eicosapentaenoic (EPA), docosahexaenoic acid (DHA) and α- linoleic acid (ALA).[7]

DHA, EPA, and their respective metabolites are known to exert a myriad of biochemical and biological 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 (Ruxton *et al*, 2005, Mayer, 2002).

Indeed, patients with steady-state sickle cell disease have abnormal red cells, characterized by elevated arachidonic acid (AA,), adrenic acid, and osbond acid and decreased linoleic acid (LA, EPA, and DHA. This suggests that the 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. In a previous retrospective study, it was observed that the greater the amounts of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the blood, the lesser te number of complications of sickle cell disease (SCD) and the higher the steady state haemoglobin level. SCD causes ischaemia-reperfusion injury and inflammation; which can be ameliorated by a metabolite of DHA that down-regulates expression of pro-inflammatory genes. [4]

Materials and methods

The study group was selected from patients aged 5–35 years with HbSS, who were undergoing regular follow-up checks at the outpatient Sickle Cell Disease Referral Clinic. The clinic is authorized to use samples for research purposes. A questionnaire was used to explain the aims of this study to the potential participants in order to obtain their permission and consent signatures.

The phenotypic characteristic was confirmed with the use of cellulose acetate electrophoresis at pH 8.5. All of the patients were receiving regular omega-3 and folate supplementation, and those 5 years of age were receiving standard oral prophylactic penicillin. All patients selected were not affected by other phenotypes, lacked the presence of other chronic diseases, had not received blood transfusions in the previous 4 months, had not received hydroxyurea treatment, or had a history of overt stroke or crisis, and were not pregnant. Seventy eight patients were randomly assigned to receive omega-3 fatty acid supplementation for at least two years. A control group of thirty five individuals were also selected randomly. A sample of venous blood (5 ml) was collected by clean venepuncture from each patient via the antecubital vein using a plastic syringe with minimum stasis, and transferred into commercially prepared concentrations of sequestrene Ethylene Di-amine Tetra-acetic Acid (EDTA) bottles. Each sample was mixed gently and thoroughly to prevent cell lysis and ensure anticoagulation.

A supplementary 5 ml venous blood sample was collected from both patients and controls and transferred into an anticoagulant bottle. An aliquot was used to determine complete blood counts (CBC) within 2 hours of collection, whereas the remainder was used to prepare haemolysate for haemoglobin electrophoresis and for reticulocyte count. Full blood count analysis done on the same day of collection using Sysmex KN-21 N, (manufactured by Sysmex corporation Kobe, Japan) a three- part auto analyzer able to determine 19 parameters per sample including haemoglobin concentration, packed cell volume, red blood cell concentration, mean corpuscular haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration, white blood cells and platelet values. A small portion of the sample was used for reticulocyte counting within a maximum of two hours from sample collection.

Results

The principal results of the study are shown in Table 1.

Table 1 The principal results of the study

Test	sample	Mean±S D	*P*.value
Hb g/dl	free of omega-3	±6.5606886	.000
	omega-3	±2.682.5939	
RBCs X103C/mm3	omega-3	±2.682.5939	.029
	free of omega-3	±2.471.3960	
Hct%	free of omega-3	4.238±22.68	
	omega-3	|2.340±21.23	.022
MCVfl	free of omega-3	8.5978±85.475	.462 *
	omega-3	9.7929±86.897	
MCHpg	free of omega-3	3.070± 28.923	.004
	omega-3	3.4850± 27.002	
MCHC%	free of omega-3	2.2247± 33.884	.555*
	omega-3	1.2247± 33.645	
Retics%	free of omega-3	3.0984± 5.686	.000
	omega-3	4.0947± 13.660	
RPI	free of omega-3	±1.17700.63758	.000
	omega-3	±2067800.77922	

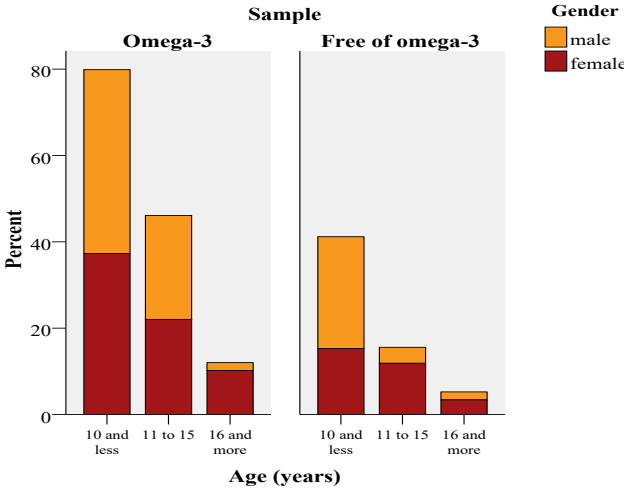

Figure 1: frequencies of sex and age groups among study population (SCD)

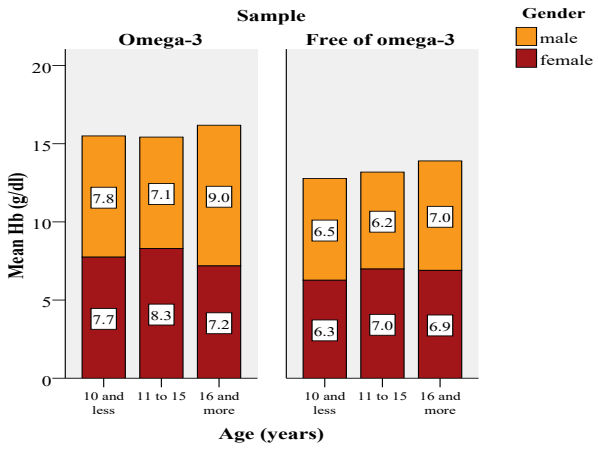

Figure 2: Mean of Hb (g/dl) of patients under omega -3 and those free of supplementation with omega-3. Two groups are compared using independent sample test; (P.value < 0.05).

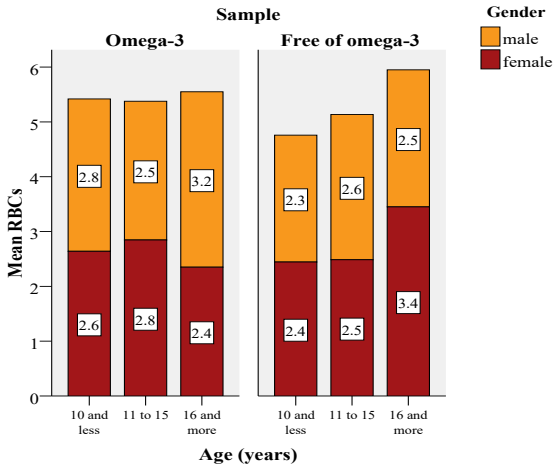

Figure 3: Mean of RBCs X103 c/Ml of patients under omega -3 and those free of supplementation with omega-3. Two groups are compared using independent sample test; (P.value < 0.05)

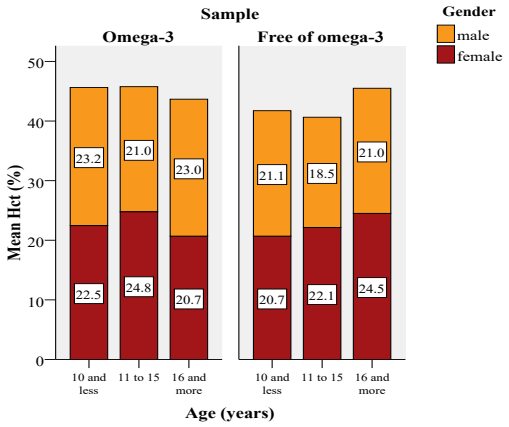

Figure 4: Mean of HCT(%) of patients under omega -3 and those free of supplementation with omega-3. Two groups are compared using independent sample test; (P.value < 0.05).

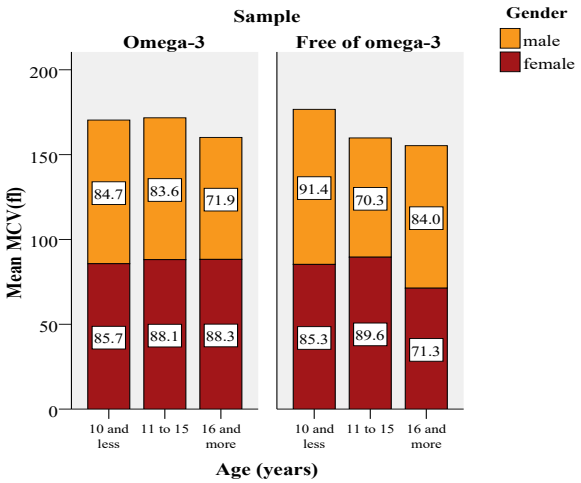

Figure 5: Mean of MCV (f l) of patients under omega -3 and those free of supplementation with omega-3. Two groups are compared using independent sample test; (P. value > 0.05)

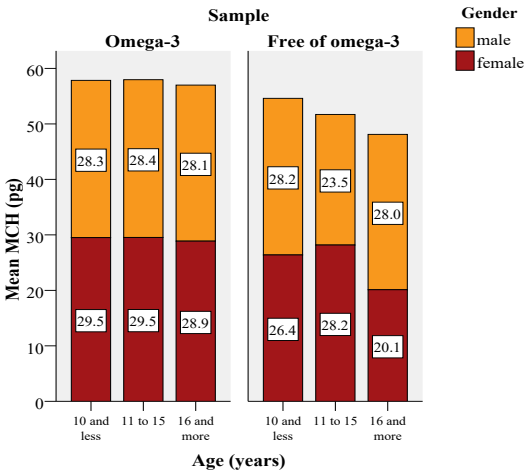

Figure 6: Mean of MCH (*pg*) of patients under omega -3 and those free of supplementation with omega-3. Two groups are compared using independent sample test; (P. value < 0.05)

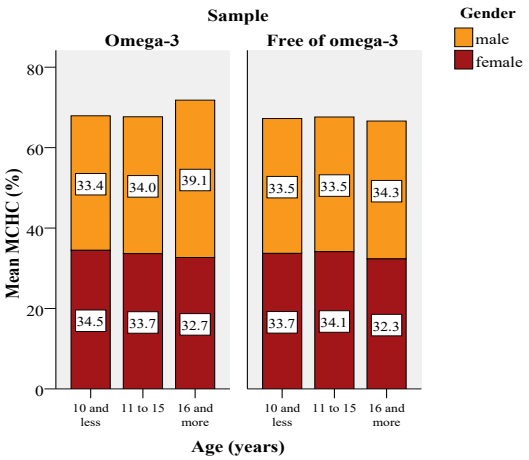

Figure 7: Mean of MCHC (%) of patients under omega -3 and those free of supplementation with omega-3. Two groups are compared using independent sample test; (P. value >0.05).

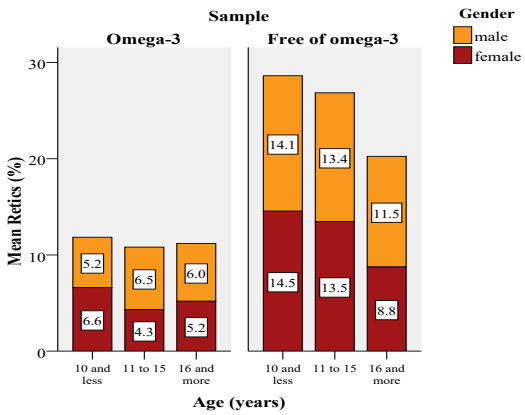

Figure 8: Mean of Reticulocytes count (%) of patients under omega -3 and those free of supplementation with omega-3. Two groups are compared using independent sample test; (P. value < 0.05)

Discussion

The presence of HbS in the homozygous state (SS) influences the red cell stability and rate of survival. Since the red cells are steadily hemolyzed a state of chronic hemolytic anemia exists.

A total of 133 subjects (mean age 10.1±4.7 years) homozygous (SS) for sickle cell anemia was studied for their hemolytic variables (Hb, Hct, TRBCs, MCV, MCH, MCHC, Retics, and RPI). Out of this total 75 were patients who were assigned to receive supplementary omega-3 and the other 38 were patients free of omega-3 supplementation who were selected randomly as a control group.

As part of the study; hemolytic variable were compared between both groups and revealed that there is a significant difference in mean Hb (g/dl) between the omega-3 group and the group free of omega-3 (P.value < 0.0001). The differences were also significant among TRBCs count, Hct %, and MCH*pg*. (P.value < 0.05). In contrast there is no difference in MCV *f* l and MCHC % levels between the study groups (P.value < 0.05) as well as sex and age variables, and this may due to the type of sickle cell anemia which is normocytic and normochromic in different populations.

In a recent study in Khartoum (Sudan), Daak et al. reveal the same result with justification: "Hemoglobin and MCH concentrations increased to the same extent in both groups after 1 year 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" (Daak et al., 2013). 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 the Sickle Cell Referral Clinic.

This study, to the best of our knowledge, is the first well powered studying bone marrow activity among the

study groups by using Retics count and RPI with significant decrease in omega-3 group compared to control group (P.value < 0.0001) and this mean decrease erythropoiesis and thus haemolysis.

In contrast with the earlier pilot studies, 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; 2) EPA is a potent inhibitor of platelet aggregation, 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. The mean percentages of DHA and EPA in the red blood cells of the Sudanese patients with sickle cell disease were lower than those in their Nigerian and British counterparts, were studied (Ren H et al.,2005). These differences are most likely a reflection of the omega-3 status of the Sudanese population, because the breast milk and red blood cells of healthy children from that country contain very low concentrations of these nutrients. (Daak *et al.,* 2013)

Conclusion

Supplementation of SCD patients with HbSS with the omega-3 fatty acids DHA and EPA was effective at reducing the frequency and severity of haemolysis, 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.

Recommendation

These findings suggest that omega-3 fatty acids can be an effective and affordable therapy for sickle cell anemia, and recommend that its use be registered and studied further under controlled trial conditions.

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