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Impact of nutrition on serum levels of docosahexaenoic acid among Omani children with autism

Yahya M. Al-Farsi M.D., Ph.D. [a], Mostafa I. Waly M.P.H., Ph.D. [b,c], Richard C. Deth Ph.D. [d], Marwan M. Al-Sharbati M.D., Ph.D. [e], Mohammed Al-Shafaee M.D. [a], Omar Al-Farsi M.Sc. [a], Maha M. Al-Khaduri M.D. [f], Samir Al-Adawi M.D., Ph.D. [e], Nathaniel W. Hodgson Ph.D. [d], Ishita Gupta M.Sc. [g], Allal Ouhtit M.Ph., Ph.D. [g,*]

[a] Department of Family Medicine and Public Health, College of Medicine and Health Sciences, Sultan Qaboos University, Al-Khoud, Sultanate of Oman
[b] Department of Food Science and Nutrition, College of Agriculture and Marine Sciences, Sultan Qaboos University, Al-Khoud, Sultanate of Oman
[c] Department of Nutrition, High Institute of Public Health, Alexandria University, Alexandria, Egypt
[d] Department of Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts, USA
[e] Department of Behavioral Medicine, College of Medicine and Health Sciences, Sultan Qaboos University, Al-Khoud, Sultanate of Oman
[f] Department of Obstetrics and Gynecology, College of Medicine and Health Sciences, Sultan Qaboos University, Al-Khoud, Sultanate of Oman
[g] Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, Al-Khoud, Sultanate of Oman

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ABSTRACT

Objectives: Autism is a lifelong neurodevelopmental disorder of early childhood. Dietary supplementation of the ω-3 fatty acid (docosahexaenoic acid [DHA]) during prenatal and postnatal life is considered a protective dietary intervention strategy to minimize the risk for autism spectrum disorder (ASD). To our knowledge, no relevant studies have been conducted in the Middle East investigating the status of DHA among children with autism during early childhood. The aim of this study was to investigate the serum levels and dietary intake status of DHA among Omani children recently diagnosed with ASD.

Methods: The present case–control study involved 80 Omani children (<5 y), 40 cases and 40 controls matched for age and sex. A semi-quantitative food frequency questionnaire was used to assess dietary intake of all the participants, while serum levels of DHA were measured using high-performance liquid chromatography.

Results: Our results showed that children with ASD had lower dietary consumption of foodstuff containing DHA, as well as lower serum levels of DHA than controls.

Conclusion: The present finding from Oman supports the view of other studies that there are low serum levels of DHA among children with ASD.

Introduction

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid of the ω-3 series, which is essential for the structural component of the central nervous system (CNS) and has direct bearing on normal development of the CNS and its counterpart, cognitive, emotional, and behavioral functioning [1–3]. Previous studies suggest that maternal milk or fish oil is a rich source of DHA and therefore low intake of DHA during pregnancy has been linked with increasing risk for developing autism among children with vulnerable predisposition [4–6]. Low intake of DHA during pregnancy appears as a physiological stress for depleting the mothers' body stores of DHA, and their subsequent offspring [7–9]. During preschool age, lower levels of DHA among children with autism was associated with exacerbation of cognitive and emotional symptoms [10–12].Conversely, ω-3 fatty acid supplementations has been shown to attenuate core and associated symptoms of autism spectrum disorders (ASD). On the whole, there is evidence suggesting that the impairments associated with ASD may be partially linked to deficits of DHA, and that supplementation with DHA may result in the improvement of

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* Corresponding author. Tel.: +96824143464.
E-mail address: aouhtit@squ.edu.om (A. Ouhtit).

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some of the debilitating symptoms commonly found in children with ASD. However, recent meta-analysis suggests that it may be premature to assume that supplementation with DHA is effective for improving the cognitive and emotional impairments associated with ASD due to a lack of studies that have employed rigorous methodology [13]. Therefore, more groundwork is needed to establish a link between DHA and ASD. Most of the previous links between DHA and ASD have been with ω-3 fatty acid supplementations and have been limited to studies from other parts of the world. There is a paucity of studies from Arabian Gulf countries. The contribution of such regions is likely to confirm whether the partial linkage between DHA and ASD is not simply an anomaly limited to cultural practice rather than integral parts of the pathogenesis of ASD. In order to fill the gap in the literature, the present study aims to investigate the status of DHA among Omani children with ASD. To our knowledge, no studies of such nature have emanated from Oman or its regional counterpart. In our quest to study correlates of ASD in Oman, we previously indicated that ASDs exhibited significant reduction in indices of folate and vitamin B_{12} [14], oxidative stress in ASD [15], elevated levels of heavy metals, and selective depletion of essential minerals [16].The present study was, therefore, conducted to investigate both the dietary intake and serum levels of DHA among Omani children recently diagnosed with autism. This study is expected to shed light on the effect of prenatal and postnatal nutrition on the DHA status of autistic children.

Participants and methods

Study participants and study setting

A case–control study approach was used for this study from July to December 2010. The study was held in the Child and Adolescent Psychiatry psychiatry outpatient clinic in the Department of Behavioral Medicine, Sultan Qaboos University Hospital, a tertiary referral hospital in Oman. All study participants were Omani nationals under the age of 5 y. The accompanying parents were briefed about the study protocol, and on a voluntary basis they signed a consent form to enroll in the study.

Power analysis was conducted for this study. A sample size of 72 children was estimated to be adequate. From this number, calculated with 10% error, either side of estimate and 95% level of confidence, the difference in DHA level was assumed to be 25%. Therefore, 80 children were recruited for this study and were divided into two equal groups: (1) the control group included children who attended the outpatient child health clinic for simple acute illnesses and had no history of neurodevelopmental disorders or any chronic diseases that appear to stem from nutritional status; (2) the case group included children newly diagnosed with autism, based on Diagnostic and Statistical Manual of Mental Disorders-IV criteria for diagnosis [17,18].

In-person interviews were scheduled for all mothers of the study participants for completing the study questionnaire, and blood samples were collected from the enrolled children to measure serum DHA levels. Blood samples were collected during regularly scheduled clinic visits by trained nurses. The following known risks for venipuncture were explained to caregivers of included children: hematoma, swelling and inflammation at the site, persistent bleeding, and vasovagal response. Special precautions were taken, such as use of sterile equipment, use of a trained nurse to perform the blood draw with a physician on call in case an adverse affect occured.

Tools of the study

Anthropometric measurements were conducted for all children according to the following standards: height was measured to the nearest 0.5 cm and weight was recorded to the nearest 0.5 kg using a precision scale. EPI-info program was used to calculate *Z* scores of anthropometric measurements using the following standards: height for age (HAZ), weight for height (WHZ), and weight for age (WAZ) as compared to the National Center of Health Statistics (NCHS/World Health Organization [WHO]) reference values. The *Z* scores (SD scores) represent how far the data are distributed (higher or lower) around the reference median. Following the classification of the WHO database on child growth, a stunted child is defined as one whose HAZ is less than −2 SD of the reference median, reflecting a long-term growth faltering. A wasted child is defined as one with WHZ less than −2 SD below the reference median, reflecting acute or recent growth disturbances, and an underweight child is defined as one with WAZ less than −2 SD of the reference median, reflecting a combination of disturbances in linear growth and body proportion.

Dietary assessment also was performed to elicit prenatal nutrition. All mothers were required to give details about their dietary habits during their pregnancy, using a Qualitative Food Frequency Questionnaire (FFQ). This measure intended to gauge usual food consumption over the previous 24 mo, with specific reference to foods rich in DHA or its precursors [7]. The FFQ was tested for its validity, reliability, and reproducibility before conducting the study. The Food Processor software version 10.2 (ESHA Research, Salem, OR, USA) was used to calculate the daily nutrient intake (total energy intake, protein, carbohydrate, and fat) as estimated from the frequency of consumption, reported portion size, and nutrient content for all foods reported by each study participant. For postnatal nutrition, mothers were asked to respond to questions related to the duration and the practice of breast-feeding as well as for the retrospective dietary intake of the children involved in the study.

Biochemical assessment of DHA

Blood was collected from participants and designated controls. Whole venous blood samples were separated by centrifugation and stored in Eppendorf tubes by freezing at −80°C and were thawed just before analysis of DHA. DHA was measured as its methyl ester, by adding 150 μL of serum to 5 mL of methanol/acetyl chloride (50:1, vol/vol); the mixture was thoroughly mixed and left at room temperature for 45 min [19]. The methylation reaction was stopped by the slow addition of 3 mL of 6% potassium carbonate solution to the mixture. After 5 min, 1 mL of hexane was added and the mixture was vigorously shaken. The hexane layer, which contained the methylated DHA, was separated and dried under nitrogen [20]. The residue was dissolved in 100 μL methanol for lipid analysis by high-performance liquid chromatography, which was carried out on a gradient system (Shimadzu C-4A, ODS, Hypersil C18), equipped with a guard column PR 8 or PR 4 and reversed-phase column. The separation of the methyl ester–derived fatty acid mixture was carried out using methanol/water (90:10, vol/vol) as a mobile phase at a flow rate of 0.5 mL/min. The eluent was monitored by UV detector at a wavelength set at 210 nm. A standard of *cis*- 4, 7, 10, 13, 16, 19- DHA methyl ester was used [20].

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Statistical analysis

Data were expressed as mean ± SD and analyzed using the GraphPad Prism statistical software for personal computer, version 5. χ^2 test was used for categorical variables. One-way analysis of variance was followed by Tukey's test, Student's unpaired *t* test and correlation coefficients (r) for continuous variables. The *P*-value < 0.05 was considered statistically significant. The study was approved by the Medical Research and Ethical Committee of the College of Medicine and Health Sciences (AGR/Food/IG/10/01), Sultan Qaboos University.

Results

The participants (cases and controls) enrolled in this study did not receive any DHA supplements or fish oil during their whole term of pregnancy. The maternal educational level (higher education and secondary school), monthly income (an average of 500–600 Omani Rials/month), and their employment status were comparable in both groups with no statistical differences ($P > 0.05$). Also, none of the enrolled children were receiving dietary supplements, vitamins, or following a special dietary regimen.

Table 1 shows the mean age for case (4.1 ± 0.9 y) and the control groups (4.1 ± 0.8 y). There were no statistical significant differences between the two age means ($t = 1.055$, $P = 0.353$). The case group had WHZ (5%), WAZ (6%), and HAZ (4%), where as the control group had lower values (WHZ 2%, WAZ 3%, and HAZ 2%, respectively). However, the difference was not statistically significant ($\chi^2 = 0.049$, $P = 0.975$). The breast-feeding duration for controls was 28.05 ± 4.58 mo, which was significantly higher than that of the cases 14.85 ± 5.26 ($t = 11.97$; $P = 0.001$).

The analysis of food group shows that the frequency of consumption (number of servings/d) of foods rich in DHA or its precursors such as fresh fish, canned fish, vegetable oils, eggs, chicken, organ meats, nuts, and beans was significantly lower among cases than controls ($P < 0.05$).

The results indicate that the case group had a significantly lower mean of total daily energy intake (kcal/d) compared with the control group (1323 ± 117.8 kcal/d and 1684 ± 101.3 kcal/d, respectively; t=14.7; $P = 0.001$) (Table 1). The differences between the two groups regarding the mean intake of α-linolenic acid (0.8 mg/d and 1.2 mg/d, respectively) was also significantly different ($t = 5.66$; $P = 0.001$).

Finally, the lowest level of the serum DHA was observed in the case group, with a mean level of 4.1 ± 0.3 μg/mL, which was significantly lower than that of the control group 8.7 ± 1.1 μg/mL, ($t = 25.52$; $P = 0.001$).

Discussion

This study investigated the changes in the serum levels of DHA in Omani children recently diagnosed with autism who were compared with their respective controls. Because no background information on the normal blood levels of DHA in Omani children were available, we compared DHA levels of the case group with their age-matched and sex-matched controls. We also compared the intake of ω-3fatty acid and α-linolenic acid in both groups.

Several studies have indicated that macronutrient and micronutrient deficiencies might be prevalent among children with autism [1–4]. A preliminary epidemiologic survey suggested that 1.4 per 10,000 Omani children (aged 0–14 y) have ASDs [17]. Al-Farsi et al [21] conducted a cross-sectional study among 128 preschool-aged Omani children with ASD in order to examine their nutritional status. The study revealed that approximately 9% of the participants were malnourished, and the most common form of malnutrition was underweight, followed by stunting, and then wasting. In another report, Al-Farsi et al found a significant decrease in the mean values of both dietary and serum levels of folate as well as vitamin B_{12} among 40 Omani children with autism compared with matched controls [14].

The underpinning causes of malnutrition among Omani children with ASD might be rooted in the early life feeding practices, as suggested by a case–control study that found that suboptimal breast-feeding practices were considerably higher among children with ASD than in children without ASD [22]. Previous studies have shown that maternal milk is a rich source of DHA [23].

Certain socioeconomic factors might play a role in a notable reduction of indices of DHA in Omani children with ASD. Despite the fact that Oman belongs to the list of "high-income economies," Omani children have been reported to have a high incidence of protein malnutrition [24]. Malnutrition and under nutrition among Omani children with ASD might be aggravated by the socioeconomic strains experienced by families caring for children with ASD. A cross-sectional survey among 150 families caring for children with ASD in Oman demonstrated that caring for these children highly affects family finances [25]. The study indicated that some caregivers may find it difficult to meet their daily expenses because of the cost of caring for children with ASD, as well as lost income that many families with children with ASD face when they need to quit their job in order to care for their disabled child.

Table 1
Anthropometric measurements, nutritional indices, and serum levels of the docosahexaenoic acid among children with autism spectrum disorders and controls, Oman, 2010

Characteristics	Cases (n = 40)	Controls (n = 40)	*P*-value
Age (y)	4.1 ± 0.9	4.1 ± 0.8	$t = 1.055$; $P = 0.353$
Z score			
WHZ (%)	5	2	
WAZ (%)	6	3	$\chi^2 = 0.049$; $P = 0.975$
HAZ(%)	4	2	
Duration of breast-feeding (mo)	14.85 ± 5.26	28.05 ± 4.58	$t = 11.97$; $P = 0.001$
Total energy intake (kcal/d)	1323 ± 117.8	1684 ± 101.3	$t = 14.7$; $P = 0.001$
α-Linolenic acid (g/d)	0.8 ± 0.2	1.2 ± 0.4	$t = 5.66$; $P = 0.001$
Serum DHA (μg/mL)	4.1 ± 0.3	8.7 ± 1.1	$t = 25.52$; $P = 0.001$

DHA, docosahexaenoic acid; HAZ, height for age; WAZ, weight for age; WHZ, weight for height
Data expressed as mean ± SD

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Because Omani mothers whose children were involved in this study had a similar daily intake of DHA-rich sources of food, such as local fatty fish or foods containing DHA precursors (α-linolenic acid) like vegetable oils, beans, and dark green vegetables, decreases in serum levels of DHA in their children might be the result of children's decreased postnatal food intake. Hence, an awareness campaign is necessary to increase the public's knowledge, especially pregnant women who should be advised to consume more food rich in DHA precursors.

Interestingly, there was a lack of knowledge among the enrolled Omani mothers about dietary sources of DHA and its health benefits. Previous studies have shown that pregnant women who used fish oil supplements boosted their DHA status [26–28]. It is recommended that the maternal diet be supplemented with DHA sources as to improve the health and well-being of women during pregnancy [29,30], as well as to reduce the risk for having a child with ASD, especially in families with a family history of autism.

Biochemical and pharmacologic studies suggest that ω-3 fatty acids might modulate the neurotransmitter metabolism and cell signal transduction in humans, and any abnormality in ω-3 fatty acid and eicosanoids metabolism may play a causal role in attention-mediated synchronization [31,32]. Our findings suggest that low intake of DHA during early childhood might be a physiological stress factor that contributes to the depletion of children's body stores of DHA, and if the growth requirement is not matched with an increased dietary intake of DHA, it might ultimately become a risk factor that synergies with other risk factors and promote the pathogenesis of autism among susceptible children.

Conclusion

Our data demonstrated that DHA serum levels were markedly low in children with ASD. This study established the temporal relationship, but not the cause and effect. There is a broad assumption in the literature that inadequate intake of food rich in ω-3 fatty acids during pregnancy and in early childhood may result in children having low DHA serum levels. If this study will withstand further scrutiny, there will be grounds to recommend that all pregnant Omani women, as well as their children, take DHA supplements. Alternatively, DHA-rich food or fortification of food with DHA to meet the daily requirement of growing children also may suffice. A public health educational program should be instrumental in reaching all sectors of Omani society.

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