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Altered IFN-γ Levels after Treatment of Epileptic Patients with Omega-3 Fatty Acids

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Abstract

Epilepsy is a frequent chronic disorder of the brain characterized by intermittent epileptic seizures caused by hypersynchronous discharge of neurons in the brain. Studies have reported the role of cytokines in the pathogenesis of epilepsy, and a number of investigations have shown decreased levels of omega-3 fatty acids in epileptic patients. We investigated differences in serum levels of two cytokines, transforming growth factor (TGF)-β and interferon (IFN)-γ, in 40 epileptic cases prior to and after treatment with omega-3 fatty acids. IFN-γ levels were significantly increased after the 16-week treatment period ($P < 0.001$). However, TGF-β levels remained unchanged ($P = 0.14$). Omega-3 fatty acid treatment may alter the immune response in epileptic patients. This should be considered in prescription of omega-3 fatty acid supplements in these patients. Future studies with larger sample sizes should verify the results of the current study.

Keywords Epilepsy · Omega-3 fatty acid · TGF-β · IFN-γ

Introduction

Epilepsy is a frequent chronic disorder of the brain with various courses and outcomes. This disorder is characterized by intermittent epileptic seizures caused by hypersynchronous discharge of brain neurons (Stafstrom and Carmant 2015). Almost 1% of the general population is affected by epilepsy (Zack and Kobau 2017). Several studies have pointed to the neuroprotective and anticonvulsant effects of omega-3 fatty acids, suggesting their potential application in the management of epilepsy (Tejada et al. 2016). Docosahexaenoic acid (DHA) is the main omega-3 fatty acid component of the brain, constituting 10–20% of overall fatty acids. Alpha-linolenic acid (ALA) and eicosapentaenoic acid (EPA) account for less than 1% of these substances in the brain (McNamara and Carlson 2006). Based on the results of investigations showing lower concentrations of omega-3 fatty acids in patients with epilepsy (DeGiorgio and Scorza 2014), omega-3 fatty acid supplements are prescribed for epileptic patients. Additionally, omega-3 fatty acids have been shown to influence the level of cytokines including tumor necrosis factor (TNF)-α, and interleukin (IL)-1β (Kang and Weylandt 2008). Cytokines may be involved in the pathogenesis of epilepsy, as several studies have shown dysregulation of cytokines in epileptic patients (Li et al. 2011). We recently conducted a triple-blind, placebo-controlled clinical trial of omega-3 fatty acid supplementation in epileptic patients and reported decreased seizure occurrence and duration as well as reduced levels of TNF-α and IL-6 following

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supplementation (Omrani et al. 2019). In the current study, we investigated differences in serum levels of two other cytokines, transforming growth factor (TGF)-β and interferon (IFN)-γ, in epileptic patients before and after treatment with omega-3 fatty acids.

Material and Methods

Patients

The current investigation was a clinical trial in which 40 patients with refractory epilepsy received omega-3 fatty acid tablets during a 16-week period. Exclusion criteria were hepatic or kidney diseases, active infectious conditions, diabetes, hypertension, dementia, mental retardation, and pregnancy or breast-feeding. Blood samples were gathered from study participants prior to and after the 16-week treatment period to test TGF-β and IFN-γ levels. Omega-3 fatty acid capsules (Zahravi Pharmaceutical Company, Tehran, Iran) that were prescribed for patients contained 120 mg of DHA and 180 mg of EPA plus vitamin E. Capsules were taken twice a day. Antiepileptic drugs were continued. The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1397.544). Written consent was obtained from all participants. The block randomization technique was used to randomize patients into two groups with equal sample sizes. Details of randomization have been described previously (Omrani et al. 2019). The independent data monitoring committee strategy was used.

Evaluation of Cytokine Levels

Five milliliters of peripheral blood was gathered from patients and used for evaluation of plasma levels of TGF-β and IFN-γ prior to and after completion of the treatment phase. Serum levels of TGF-β and IFN-γ were evaluated by commercial enzyme-linked immunosorbent assay (ELISA) kits. In brief, 100 µl of standard or sample was first added to each well of the microplate, and the microplate was then incubated for 2 h at room temperature. Next, 100 µl of the prepared biotin antibody was added to each well. After 1 h incubation at room temperature, 100 µl of prepared streptavidin solution was added to each well. After 45 min incubation at room temperature, substrate reagent was added. Further steps were incubation at room temperature for 30 min, addition of 50 µl of Stop Solution, and immediate reading at 450 nm.

Table 1 Characteristics of study participants

Variable	Value
Female/male [no. (%)]	25 (62.5%) / 15 (37.5%)
Age (mean ± SD, years)	36.66 ± 2.8
Age range (years)	21–58
Age at onset (mean ± SD, years)	28 ± 8.6
Disease duration (mean ± SD, years)	8.18 ± 4.1

Statistical Analysis

The quantitative data are described as the mean (± standard error). A paired-samples *t* test was used for evaluation of differences in cytokine levels before and after treatment.

Results

Patient data are displayed in Table 1.

IFN-γ levels were significantly increased after the treatment period ($P < 0.001$). However, TGF-β levels remained unchanged ($P = 0.14$). Figure 1 shows the results of assessment of cytokine levels before and after treatment. Table 2 shows the mean values of cytokine levels at two time points, before and after treatment.

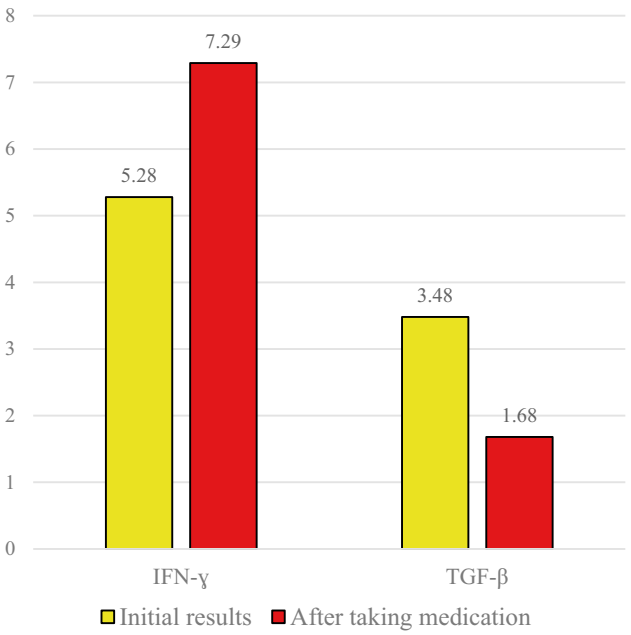

Fig. 1 Results of assessment of cytokine levels before and after treatment

Table 2 Mean values (± standard deviation) of cytokine levels at two time points

	IFN-γ level (pg/ml)	*P* value	TGF-β level (pg/ml)	P value
Initial results vs. after taking medication	5.28 (9.32) vs. 7.29 (16.32)	< 0.001	3.48 (1.4) vs. 1.68 (1.38)	0.14

Discussion

Omega-3 fatty acids have been administered for epileptic patients for a relatively long time. However, the results of studies are not in harmony. In a phase II randomized crossover trial, DeGiorgio et al. reported efficacy of low-dose fish oil in decreasing seizure frequency compared with placebo (DeGiorgio et al. 2015). However, Bromfield et al. detected no superiority of polyunsaturated fatty acid supplementation compared to placebo as adjunctive therapy for refractory epilepsy (Bromfield et al. 2008). A recent systematic review of the available literature regarding administration of omega-3 fatty acids in epileptic patients revealed controversial findings as to the beneficial effects of these supplements in these patients (Pourmasoumi et al. 2018). Independent of the effects of omega-3 fatty acids on clinical aspects of epileptic patients, these substances may alter the immune responses in these patients. Animal studies have shown the effects of omega-3 fatty acids on cytokine levels and modulation of the course of autoimmune disorders. For instance, these substances were found to enhance TGF-β1 levels in the spleens of mice. Alterations in membrane fatty acid composition have been suggested to influence immune responses and gene expression patterns in the course of systemic lupus erythematosus (Fernandes et al. 1994). Moreover, consumption of omega-3 polyunsaturated fatty acids has been reported to decrease expression of receptors for IFN-γ (Feng et al. 1999). In the current study, we verified this speculation through the observed higher levels of IFN-γ in these patients after a 16-week treatment period. Sinha et al. reported detectable levels of this cytokine in about 20% of epileptic patients in the immediate post-ictal period, and also reported decreased concentrations of this cytokine in seizure-free periods. In addition, they showed higher levels of IFN-γ in epileptic patients compared with healthy subjects (Sinha et al. 2008). Our results are not consistent with theirs, as we found higher levels of IFN-γ and lower seizure frequency (as reported in our recent study; Omrani et al. 2019) following treatment with omega-3 fatty acids. Based on the role of IFN-γ in the breakdown of the blood–brain barrier and the pro-inflammatory effect of this cytokine (Sonar et al. 2017), our results might indicate an altered immune response in epileptic patients following treatment with omega-3 fatty acids. However, Sinha et al., when reporting detectable levels of both pro-inflammatory and anti-inflammatory cytokines in epileptic patients, speculated on a possible pleiotropic characteristic of the cytokines, wherein different cytokines may exert both pro-inflammatory and anti-inflammatory effects based on the environmental situation and the condition of cell induction (Sinha et al. 2008). A more recent study in animal models of pilocarpine-induced epilepsy demonstrated alterations in the quantities of microglial phenotypes following injection of IL-4/IFN-γ. Such alterations were accompanied by improvements in epilepsy outcomes (Li et al. 2017). Taken together, these findings could lead one to speculate that the ratio of cytokines and the timing of their alterations affect the cell phenotypes and clinical outcomes. Consequently, we propose a longitudinal evaluation of cytokine levels in epileptic patients to investigate their fluctuations during the disease course and the correlation between their levels. Such studies could unravel the complex interactions between pro-inflammatory and anti-inflammatory cytokines in the context of epilepsy.

We found no significant difference in TGF-β levels before and after treatment with omega-3 fatty acids. The contribution of this cytokine in epilepsy has been confirmed in animal studies, which reported its upsurge in the neurons of amygdala-kindled animals (Plata-Salaman et al. 2000). In addition, the levels of this cytokine were upregulated in astrocytes from the hippocampus after status epilepticus (Aronica et al. 2000). However, the significance of TGF-β in human epileptogenesis is not clear. Although omega-3 fatty acids have increased expression of this cytokine in ovarian cancer cells (Sharma et al. 2009), alimentary omega-3 lipids have been shown to suppress mRNA and protein expression of TGF-β in an animal model of systemic lupus erythematosus (Chandrasekar et al. 1995). Consequently, it can be deduced that the impacts of omega-3 fatty acids on TGF-β levels depend on the cell type and disease condition.

In summary, we demonstrated higher levels of IFN-γ and unchanged levels of TGF-β after a 16-week treatment with omega-3 fatty acids in epileptic patients. Our study is limited in that we did not assess plasma levels of DHA and EPA in the study subgroups before and after treatment. As the sample size of the study is small, the results should be confirmed in other studies. Future studies are needed to evaluate the effects of these supplements in modulating the immune response in patients with epilepsy.

Author Contributions SGF and MT wrote the manuscript and revised it. MAO and MH analyzed the data. AS, MR and BMH performed the experiment and designed the tables. All authors approved the final manuscript.

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Data Availability The data sets generated and analyzed during the study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1397.544). All methods were performed in accordance with the relevant guidelines and regulations.

Competing Interests The authors declare that they have no conflict of interest.

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