



Effects of omega-3 fatty acids on serum levels of T-helper cytokines in children with asthma



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ABSTRACT

Background: There has been a considerable interest in the potential therapeutic value of dietary supplementation with ω -3 fatty acids in patients with asthma.

Objectives: This cross-sectional study was designed to identify the effect of ω -3 fatty acids on symptom score, pulmonary function and serum T-helper cytokine concentrations in children with mild to moderate persistent asthma.

Methods: A total of 39 patients among 50 volunteers completed this 3-month study. They took a soft gel capsule containing 180 mg EPA and 120 mg DHA daily. Pulmonary function was evaluated in 28 eligible patients by spirometry, and serum levels of Th1, Th2, Th9, Th17 and Th22 cytokines were measured by multiplex cytometric bead assay before and after treatment.

Results: After treatment with ω -3, symptom score improved in 28 (72%) patients. The results of spirometry showed remarkable improvement in FEV₁/FVC ($P = 0.044$) and PEF ($P < 0.0001$) after treatment, but considering a cut-off of 80%, real improvement was observed in 32% of patients with PEF $< 80\%$ which raised above the cut-off after ω -3 treatment ($P = 0.004$) whereas, FEV₁/FVC changes were above the cut-off value in 89% of the patients. After treatment, IL-17A and TNF- α levels decreased significantly (both $P = 0.049$).

Conclusion: Oral administration of natural anti-inflammatory products such as ω -3 is a promising complementary approach to managing asthma.

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1. Introduction

Allergic asthma is a chronic inflammatory disease in which Th2-derived cytokines (IL-4, IL-5 and IL-13) and IL-9, IL-17 and IL-22 have key roles in pathogenesis [1]. During this inflammatory process, arachidonic acid release leads to the production of mediators which in turn contribute in bronchoconstriction, edema and increased mucus associated with the symptoms of asthma [2].

Omega-3 (ω -3) is a group of polyunsaturated fatty acids (PUFAs) which are essential for human cells and should be supplied by the diet. In contrast to n-6 PUFAs, which are derived from linoleic acid, linolenic acid is the source of n-3 series fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). N-3 fatty acids are commonly found in marine oils, and fish oil is considered the best animal source. These fatty acids compet-

itively inhibit the formation of prostaglandins and leukotrienes derived from arachidonic acid [3].

Observational studies have reported a lower rate of asthma in populations that consume large amounts of cold-water fish rich in ω -3 PUFAs [4,5]. The low incidence of asthma in Eskimos might be attributable to their nutritional regimen, which contains large amounts of oily fish [6]. Dietary supplementation with ω -3 in patients with asthma has also shown encouraging results [7,8]. Nevertheless, human-based evidence supporting the beneficial effects of dietary ω -3 fatty acids in patients with asthma is limited. This study was designed to evaluate symptom score, pulmonary function and Th1, Th2, Th9, Th17 and Th22 cytokine levels in children with persistent asthma after a 3-month intervention with ω -3 PUFAs.

2. Methods

2.1. Patient selection

After approval of the study protocol by our University Ethics Committee in compliance with Iranian Registry of Clinical Trials

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(IRCT2014111419940N1), which addresses the guidelines for research with human subjects, to recruit volunteers, an announcement was distributed in allergy clinics affiliated with Shiraz University of Medical Sciences. During 2014, 50 families who had a child with proven persistent asthma according to standard criteria [9], registered to participate in the study. Patients with a history of allergy to seafood and those under treatment with anti-interleukins and oral corticosteroid were excluded from the study.

After the patients' parents provided their written informed consent, information about demographic data including age, sex, weight, height, and family history of atopy was collected. The patients were scheduled to take a soft gel fish oil capsule (Best Formulations Inc., Los Angeles, CA, USA) containing 180 mg EPA and 120 mg DHA daily for 3 consecutive months. The participants continued their usual diet and previously prescribed asthma control regimen with inhaled corticosteroids during the intervention period.

At the beginning of the study and after 3 months of treatment, blood samples were obtained from each patient and serum samples were isolated and stored at -20°C for cytokine assays. The patients were also evaluated for subjective symptoms (nocturnal cough, nocturnal wheeze and daytime wheeze). Improvement in subjective symptoms after treatment with ω -3 was assessed on a score of 0–3. Patients with a 2-point change in score were considered to show substantial improvement, a 1-point change was considered partial improvement, and those with no change in score were considered to show no improvement [10].

2.2. Pulmonary function test

Pulmonary function was tested in eligible patients by an expert physician before and after treatment with the ω -3 dietary supplement. Respiratory volumes including forced expiratory volume in 1 s (FEV_1), ratio of FEV_1 to forced vital capacity (FEV_1/FVC) and peak expiratory flow (PEF) were evaluated by spirometry (Cosmed, Rome, Italy) in eligible patients, and values less than 80% of the predicted value were considered abnormal [11].

2.3. Cytokine assay

Serum levels of cytokines belonging to the Th1 (IFN- γ , IL-1 β , TNF- α , IL-2 and IL-12 p70), Th2 (IL-4, IL-5, IL-6, IL-10 and IL-13), Th17 (IL-17A), Th9 (IL-9) and Th22 (IL-22) groups were quantified by multiplex bead-based assay with a commercial kit (eBioscience, Vienna, Austria) according to the manufacturer's directions. Briefly, a mixture of antibody-coated beads for each cytokine to be measured was incubated with the serum samples or standards. After capturing of the cytokines by these FITC-labeled beads which could be differentiated by their sizes and fluorescent intensities, a biotin-conjugated secondary antibody mixture was added and then PE-conjugated streptavidin was added as detector. The results were visualized with a FACSCalibur flow cytometer (eBioscience, Santa Cruz, CA, USA) and data were evaluated with FlowCytomix Pro-3.0 software (eBioscience).

2.4. Statistical analyses

The Shapiro-Wilk test was used to evaluate normality of the data, and variables that did not show a normal distribution are expressed as medians with ranges and percentiles. Cytokine levels and spirometry indices are presented as the median and interquartile range (IQR), and P values were generated for group comparisons between pre- and post-treatment values (Δ) with the Wilcoxon rank-sum test. The McNemar test was used to compare qualitative changes in spirometry parameters before and after the intervention based on a cut-off of 80%. Spearman rank correlation

was calculated to assess the association of changes in spirometry indices or alterations in serum cytokine levels with age or body mass index (BMI). The Mann-Whitney U test was used to evaluate the correlation between these changes and sex, family history or improvement in symptom scores. All statistical analyses were done with SPSS version 23 (SPSS Inc., Chicago, IL, USA) and a two-sided P value equal to or less than 0.05 was considered statistically significant.

3. Results

Of 50 volunteers initially recruited for the study, 2 patients were excluded because of abdominal discomfort after taking the first doses of fish oil, 4 patients were excluded because of noncompliance with treatment, and 5 patients missed their post-treatment appointment for blood testing. A total of 39 patients (15 girls and 24 boys) with mild to moderate persistent asthma, aged between 4 and 14 years (mean $\pm$ SD 8 ± 2.6 years) and with a BMI between 13 and 21.6 kg/m^2 (mean $\pm$ SD $16 \pm 2 \text{ kg/m}^2$), completed the study. Of these 39 children, 17 (44%) had a family history of atopy.

Two-point improvements in symptom score were observed in 28 (72%) patients after treatment with ω -3, and one-point improvements in 6 (15%). No change was observed in the 5 (13%) remaining patients.

The results of spirometry showed remarkable improvement in FEV_1/FVC ($P = 0.044$) and PEF ($P < 0.0001$) after the intervention, but considering a cut-off of 80%, real improvement was observed in 9 out of 28 patients (32%) with $\text{PEF} < 80\%$ which raised above the cut-off after ω -3 treatment ($p = 0.004$) whereas, FEV_1/FVC changes were above the cut-off value in 89% of the patients (Table 1).

Comparisons of the mean and median levels of 13 cytokines before and after ω -3 treatment are summarized in Table 2. As shown, IL-17A and TNF- α levels were significantly lower after treatment (both $P = 0.049$). The total amounts of cytokines in each helper T cell subgroup, and the ratios of other subgroups to Th1 [$\text{Th2}/\text{Th1}$, $(\text{Th2} + \text{Th17})/\text{Th1}$, $(\text{Th2} + \text{Th17} + \text{Th9})/\text{Th1}$, $(\text{Th2} + \text{Th17} + \text{Th9} + \text{Th22})/\text{Th1}$] did not differ after treatment compared to pre-treatment values.

Table 3 shows the correlations between spirometry indices and cytokine levels, and the relations between changes before and after treatment with ω -3. Significant negative associations were found between $\Delta\text{FEV}_1\%$ and $\Delta\text{IL-1}\beta$ ($\rho = -0.4$, $P = 0.035$), $\Delta\text{FEV}_1\%$ and $\Delta\text{IL-6}$ ($\rho = -0.393$, $P = 0.038$), and $\Delta\text{PEF}\%$ and $\Delta\text{IL-22}$ ($\rho = -0.397$, $P = 0.047$), whereas a positive association was seen between $\Delta\text{FEV}_1/\text{FVC}\%$ and $\Delta\text{IL-13}$ ($\rho = 0.484$, $P = 0.009$).

There were no associations between considerable or partial improvement in symptom score and changes in lung function or cytokine levels.

No associations were observed between changes in spirometry indices or cytokine levels before and after the intervention and age, sex, BMI, symptom score or family history of atopy, except for $\Delta\text{FEV}_1\%$, which showed a negative association ($\rho = -0.496$, $P = 0.007$), and $\Delta\text{IL-5}$, which was directly associated with increasing age ($\rho = 0.425$, $P = 0.007$).

4. Discussion

The growing number of people with asthma in recent decades reflects the massive impact of environmental factors on disease induction. Unlike rapid epigenetic changes in response to environmental factors, the development of genetic changes generally requires a long period of time [12].

Air pollution and diet play documented roles in the induction and exacerbation of asthma [13,14]. Reducing dietary antioxidants

Table 1

(a) Spirometry indices in children with persistent asthma before and after the treatment with ω -3 (n = 28). (b) Distribution of spirometry indices higher than and lower than the 80% cut-off value.

(a)								
Spirometry indices (%)	Testing time	Range	Mean $\pm$ SD	Median (IQR)	P value			
FVC	Before	68–137	87.7 $\pm$ 18.92	86 (21)	0.089			
	After	73–137	81.96 $\pm$ 14.94	91.5 (17.25)				
FEV ₁	Before	75–112	90.11 $\pm$ 12.97	92.5 (20.75)	0.736			
	After	71–122	83.25 $\pm$ 15.11	92 (20)				
FEV ₁ /FVC	Before	71–100	111.14 $\pm$ 12.33	99 (5)	0.044			
	After	75–100	108.22 $\pm$ 8.74	98.5 (13.75)				
PEF	Before	57–96	71.92 $\pm$ 14.94	71 (15.75)	0.0001			
	After	44–120	69.11 $\pm$ 22.29	82 (17.25)				
(b)								
Spirometry indices (%)								
	FVC		FEV ₁		FEV ₁ /FVC		PEF	
	n (%)		n (%)		n (%)		n (%)	
After	<80	$\geq$ 80	<80	$\geq$ 80	<80	$\geq$ 80	<80	$\geq$ 80
Before								
<80	4 (14.3)	4 (14.3)	2 (3.6)	4 (14.3)	1 (3.6)	0 (0.0)	10 (35.7)	9 (32.1)
$\geq$ 80	1 (3.6)	19 (67.9)	1 (7.1)	21 (89.3)	2 (7.1)	25 (89.3)	0 (0.0)	9 (32.1)
P value	0.375		0.375		0.50		0.004	

IQR: interquartile range.

and vitamins as well as increasing the ratio of n-6/n-3 PUFAs in the diet are reportedly associated with asthma [14]. Although in utero or early exposure to ω -3 fatty acids reduces childhood asthma [14], its effectiveness in the treatment of established asthma in humans is still controversial [15,16]. Although current strategies for asthma management are highly effective, the administration of natural supplements like ω -3 is a potentially promising approach to reduce the side effects of pharmacological treatments and prevent tachyphylaxis [17].

There is growing evidence of the effectiveness of ω -3 in improving pulmonary function and symptoms in patients with asthma. Nagakura et al. found that the daily consumption of ω -3 PUFAs (84 mg EPA and 36 mg DHA) had beneficial effects on clinical symptoms of children with asthma over a 10-month period [8]. Emelyanov et al. also observed a significant decrease in daytime wheezing and increased morning PEF in adult patients with atopic asthma who took two capsules containing 50 mg ω -3 PUFAs twice daily for 8 weeks [18]. These results are consistent with our finding that symptom score improved in 28 of our 39 patients (72%). Improvements in PEF% were also observed in 9 of our 28 (32%) patients eligible for spirometry, although other indices showed no significant change after the 3-month intervention with ω -3. Brannan et al. recently showed that treatment for 3 weeks with ω -3 did not improve airway responses to mannitol in patients with mild to moderate asthma [19]. Their results indicate that despite the reduction in airway inflammation, short-term treatment with ω -3 may not be sufficient to improve lung function in patients with asthma.

The anti-inflammatory effects of ω -3 PUFAs have been documented in chronic inflammatory diseases such as rheumatoid arthritis [20], but further evidence is needed to clarify their effects in asthma. The beneficial effect of ω -3 in reducing airway inflammation can be explained by competitive inhibition of phospholipase-A2 activity by arachidonic acid, which successively blocks the production of proinflammatory eicosanoids such as prostaglandin E2 and leukotriene B4. As precursors of bioactive anti-inflammatory molecules such as resolvins, protectins and maresins, ω -3 fatty acids restrict neutrophilic and eosinophilic infiltration. Moreover, proinflammatory cytokines have been

Table 2

Means and medians of cytokines (pg/mL⁻¹) in serum of children with persistent asthma before and after the treatment with ω -3 (n = 39).

Cytokine	Sampling time	Range	Mean $\pm$ SD	Median (IQR) ^a	P value
IL-12	Before	0.75–5635.79	152.73 $\pm$ 901.87	0.75 (0.00)	0.08
	After	0.75–4946.57	132.52 $\pm$ 791.64	0.75 (0.00)	
IFN- γ	Before	0.80–74797.46	1954.92 $\pm$ 11972.08	0.80 (0.00)	0.123
	After	0.80–74797.46	1942.39 $\pm$ 11973.58	0.80 (0.00)	
IL-1 β	Before	2.10–4162.51	118.31 $\pm$ 665.54	2.10 (6.33)	0.075
	After	2.10–3856.28	107.38 $\pm$ 616.66	2.10 (0.00)	
TNF α	Before	1.60–17908.09	478.88 $\pm$ 2865.49	1.60 (0.00)	0.049*
	After	1.60–14521.72	386.05 $\pm$ 2323.94	1.60 (0.00)	
IL-2	Before	7.81–9758.32	365.72 $\pm$ 1557.8	60.32 (139.86)	0.578
	After	8.20–9434.20	360.37 $\pm$ 1503.02	64.07 (145.410)	
IL-10	Before	0.95–20626.92	555.40 $\pm$ 3299.96	0.95 (8.81)	0.064
	After	0.95–15378.14	413.14 $\pm$ 2460.34	0.95 (0.00)	
IL-6	Before	0.60–6943.47	183.07 $\pm$ 1111.39	0.60 (0.00)	0.109
	After	0.60–5379.12	141.48 $\pm$ 861.02	0.60 (0.00)	
IL-13	Before	2.25–6123.19	217.60 $\pm$ 973.35	49.80 (21.41)	0.267
	After	2.25–5737.58	203.72 $\pm$ 911.84	49.22 (31.52)	
IL-4	Before	7.65–9839.91	291.17 $\pm$ 1572.29	10.40 (29.07)	0.097
	After	2.39–8074.48	239.02 $\pm$ 1290.13	10.40 (21.32)	
IL-5	Before	0.80–58982.16	1594.74 $\pm$ 9435.5	0.80 (34.36)	0.55
	After	0.80–58982.16	1583.03 $\pm$ 9435.54	0.80 (37.19)	
IL-17A	Before	1.25–10613.20	367.82 $\pm$ 1691.12	1.25 (194.86)	0.049*
	After	1.25–9031.26	313.35 $\pm$ 1439.72	1.25 (144.05)	
IL-9	Before	0.43–4794.54	185.58 $\pm$ 768.49	15.00 (91.38)	0.58
	After	0.56–5062.18	187.22 $\pm$ 808.88	18.61 (72.93)	
IL-22	Before	21.65–11716.21	795.25 $\pm$ 1851.83	459.40 (302.13)	0.286
	After	21.65–10184.92	803.79 $\pm$ 1573.17	477.42 (315.93)	

IQR: interquartile range, *: Significant with P < 0.05.

reported to be downregulated when nuclear factor kappa-B (NF- κ B) activation is inhibited after ω -3 consumption [21].

The impact of proinflammatory cytokines has been suggested in the pathogenesis of allergic asthma and targeting of these mediators can potentially be considered as new approaches in the treatment of asthma [22]. Our results also showed that IL-17 and TNF- α levels decreased significantly in patients who consumed the ω -3 fatty acid supplement for 3 months. A role for IL-17 has been described in pulmonary inflammation, mucus production and airway hyperresponsiveness of asthma [23], and there is also evidence that elevated levels of IL-17 correlate with the severity of asthma [1]. The ability of resolvin E1, a product of ω -3 fatty acids, to reduce IL-17 production was recently described in a mouse model of asthma [24]. Because IL-17 stimulates the production of various inflammatory cytokines such as IL-6, IL-1 α and TNF- α , it

Table 3Correlations between spirometry indices and cytokine levels, and statistical relationships of changes before and after the treatment with ω -3.

		IL-12	IFN- γ	IL-1 β	TNF- α	IL-2	IL-10	IL-6	IL-13	IL-4	IL-5	IL-17	IL-9	IL-22
<i>Pre-treatment</i>														
FVC%	<i>rho</i>	−0.156	−0.385*	−0.254	−0.165	−0.313	−0.234	−0.287	−0.249	−0.239	−0.134	−0.168	−0.367	−0.225
	<i>P</i>	0.427	0.043	0.192	0.401	0.105	0.231	0.138	0.2	0.22	0.497	0.392	0.055	0.25
FEV ₁ %	<i>rho</i>	−0.053	−0.391*	−0.193	−0.167	−0.188	−0.155	−0.148	−0.164	−0.262	−0.049	−0.134	−0.27	−0.13
	<i>P</i>	0.79	0.039	0.326	0.396	0.338	0.432	0.452	0.406	0.179	0.806	0.498	0.165	0.509
FEV ₁ /FVC%	<i>rho</i>	0.236	0.204	0.327	0.156	0.332	0.306	0.274	0.213	0.151	0.174	0.236	0.284	0.395*
	<i>P</i>	0.227	0.299	0.089	0.427	0.085	0.113	0.159	0.277	0.443	0.375	0.227	0.144	0.038
PEF%	<i>rho</i>	−0.082	−0.087	−0.049	−0.243	−0.046	−0.037	−0.124	0.052	0.073	−0.094	−0.199	0.021	0.029
	<i>P</i>	0.679	0.661	0.804	0.214	0.818	0.851	0.528	0.791	0.711	0.635	0.309	0.915	0.885
<i>Post-treatment</i>														
FVC%	<i>rho</i>	−0.127	−0.092	0.001	−0.071	−0.097	−0.045	−0.011	−0.049	−0.249	−0.153	−0.161	0.053	0.075
	<i>P</i>	0.519	0.642	0.995	0.721	0.622	0.822	0.954	0.804	0.202	0.437	0.413	0.789	0.706
FEV ₁ %	<i>rho</i>	−0.057	0.101	0.104	−0.044	−0.034	0.037	0.091	0.103	−0.147	0.01	0.011	0.028	0.201
	<i>P</i>	0.772	0.61	0.6	0.826	0.864	0.853	0.647	0.603	0.456	0.959	0.954	0.886	0.305
FEV ₁ /FVC%	<i>rho</i>	0.109	0.371	0.259	0.111	0.322	0.218	0.198	0.326	0.252	0.304	0.446*	−0.001	0.122
	<i>P</i>	0.582	0.052	0.183	0.574	0.095	0.264	0.313	0.09	0.196	0.116	0.017	0.997	0.536
PEF%	<i>rho</i>	−0.03	−0.051	0.118	−0.028	0.298	0.146	−0.028	0.118	0.107	0.197	0.18	0.099	−0.152
	<i>P</i>	0.881	0.796	0.548	0.889	0.124	0.459	0.888	0.551	0.589	0.315	0.36	0.616	0.44
		Δ IL-12	Δ IFN- γ	Δ IL-1 β	Δ TNF- α	Δ IL-2	Δ IL-10	Δ IL-6	Δ IL-13	Δ IL-4	Δ IL-5	Δ IL-17	Δ IL-9	Δ IL-22
Δ FVC%	<i>rho</i>	−0.11	−0.283	−0.359	−0.075	−0.166	−0.147	−0.302	−0.16	−0.324	−0.174	−0.198	−0.338	−0.216
	<i>P</i>	0.576	0.144	0.06	0.705	0.399	0.454	0.119	0.415	0.093	0.377	0.313	0.079	0.269
Δ FEV ₁ %	<i>rho</i>	−0.143	−0.305	−0.400*	−0.105	−0.137	−0.15	−0.393*	0.017	−0.239	−0.25	−0.296	−0.288	−0.312
	<i>P</i>	0.468	0.114	0.035	0.595	0.486	0.447	0.038	0.93	0.22	0.199	0.126	0.137	0.106
Δ FEV ₁ /FVC%	<i>rho</i>	0.031	0.259	0.06	0.116	0.096	0.039	0.031	0.484**	0.337	−0.11	−0.183	0.125	0.142
	<i>P</i>	0.876	0.184	0.761	0.558	0.625	0.844	0.874	0.009	0.079	0.577	0.352	0.526	0.472
Δ PEF%	<i>rho</i>	0.149	−0.032	0.076	0.278	0.294	0.2	0.01	0.232	0.156	−0.044	−0.144	0.029	−0.379*
	<i>P</i>	0.448	0.872	0.699	0.151	0.128	0.307	0.959	0.235	0.428	0.825	0.465	0.883	0.047

rho: Spearman rank correlation coefficient, *: Significant with $P < 0.05$, **: Significant with $P < 0.01$.

appears that ω -3 reduces inflammation in part by decreasing the level of IL-17 [25]. TNF- α , one of the downstream cytokines induced by IL-17, plays an important role in the pathophysiology of asthma by inducing Th17 and Th2 responses [26]. Novak et al. found that TNF- α was downregulated in LPS-stimulated murine macrophages after treatment with ω -3 [27]. As previously has been explained by Clader, ω -3 appears to prevent I κ B phosphorylation, which in turn inhibits NF- κ B activation, which acts as a transcription factor for some inflammatory cytokines such as TNF- α [28]. Furthermore, ω -3 consumption in the present study also led to slight decreases in other NF- κ B target cytokines such as IL-1 β , IL-6 and IFN- γ (Table 2) [29]. In this connection, five members of the NF- κ B family form a number of homo- or heterodimers, each of which can regulate one or more target genes in cooperation with other transcription factors [30].

We observed a slight decrease in each of the Th2-driven cytokines (IL-4, IL-5, IL-6 and IL-13) after ω -3 consumption, but found no significant reduction in the combined levels of Th2 cytokines even when the levels of Th9-, Th17- and Th22-related cytokines were considered. Although concurrent imbalances in Th1/Th2 and Th17/Treg with increased Th2 and Th17 cell activity are reportedly involved in the pathogenesis of allergic asthma [31], cooperation between Th1 and Th2 cytokines should not be ignored in this process [32].

We also observed negative associations between FEV₁% and all of the cytokines tested in our patients, and this association was significant for IFN- γ after ω -3 consumption ($\rho = -0.391$, $P = 0.039$) (Table 3). These results are consistent with the previous finding of increased levels of IFN- γ in acute exacerbation and severe asthma [32]. We noted small increases in the correlation coefficients between FEV₁% and most of the cytokines we tested after the dietary ω -3 intervention. In contrast, Δ FEV₁% showed negative associations with Δ IL-1 β ($\rho = -0.4$, $P = 0.035$) and Δ IL-6 ($\rho = -0.393$, $P = 0.038$). In other words, improved FEV₁% was

associated with reduced levels of the proinflammatory cytokines IL-1 β and IL-6 after ω -3 consumption.

Although IL-22 serum levels showed no significant changes after ω -3 consumption (Table 2), we observed a negative association between Δ IL-22 and Δ PEF% (Table 3). Since both proinflammatory and anti-inflammatory properties have been reported for IL-22 [33], our results suggest that ω -3 helped to improve lung function by reducing lung inflammation. Considering the complex interrelated connections among cytokines, however, we cannot attribute the improvements in lung function to IL-22 alone [34].

The cytokine IL-13 plays a central role in the pathogenesis of allergic asthma by inducing IgE synthesis, mucus hypersecretion, airway hyperreactivity and fibrosis, which may reduce the FEV₁/FVC% ratio [35]. The direct correlation between Δ FEV₁/FVC% and Δ IL-13 in the present study (Table 3) is thus hard to explain, because more than 89% of the changes in FEV₁/FVC% were within the normal range before and after ω -3 treatment (Table 1b).

We found an inverse association between Δ FEV₁% and age ($\rho = -0.496$, $P = 0.007$); however, there was no correlation between FEV₁% and age before or after treatment ($\rho = 0.207$, $P = 0.291$ and $\rho = 0.025$, $P = 0.900$, respectively). The age-dependent reduction in lung function is a typical process in older people because of physical changes in the body [36]. Among participants in the present study, FEV₁% changes after ω -3 consumption were smaller in younger children, which may indicate that the dose of ω -3 was insufficient for older children with a larger BMI. Although a negative association between FEV₁% and BMI was previously reported in obese adults [37], all of the children in the present study had a BMI less than 25. Nevertheless, we noted a direct association between age and BMI among our patients ($\rho = 0.53$, $P = 0.001$).

Our results showed a direct association between Δ IL-5 levels and age ($\rho = 0.425$, $P = 0.007$). Although age-related increases

in IL-5 production are believed to be involved in viral-induced exacerbations of asthma in children [38], we found no significant correlation between IL-5 levels and age before or after treatment ($\rho = -0.227$, $P = 0.165$ and $\rho = -0.020$, $P = 0.903$, respectively). Although further reductions in IL-5 levels were seen in older children, changes in other cytokines may also be needed to improve lung function. It is thus plausible that a higher dose of ω -3 is needed for the lungs to regain normal activity.

In conclusion, our data document the beneficial effects of a 3-month course of ω -3 dietary supplementation on asthma symptoms and lung function. In addition, dietary ω -3 reduced serum levels of two crucial proinflammatory cytokines, TNF- α and IL-17, in patients with childhood asthma. A potential limitation of our study was the lack of a placebo-controlled group. Olive oil has been used as a placebo in similar studies [15,17], but because it has some immunomodulatory effects [39,40], this choice of placebo can lead to potentially misleading results.

To verify participant compliance with the scheduled ω -3 consumption, and to take into account the possibility of individual differences in ω -3 absorption and metabolism, it would be better to measure the levels of PUFAs in the erythrocyte membrane of each patient after the treatment period [41]. According to Li et al., changes in serum cytokine levels were consistent with the changes observed in bronchoalveolar lavage (BAL) fluid [42], so evaluating cytokine levels in BAL, which is considered a safe research tool [43], would have been a better choice to identify changes in local immune responses in the lung mucosa after our intervention. However, we were unable to obtain consent from the patients' parents for this procedure. Although helper T cells are the main source of cytokine production, epithelial cells and smooth muscle cells also contribute to cytokine secretion in the lung [44].

To verify the beneficial effects of ω -3 in patients with asthma, further studies will be needed with larger numbers of patients, broader age ranges, different severities of asthma, longer treatment periods and different doses of ω -3.

However there is no report about the life-threatening side effects of n-3 USFAs [45], like any other products, ω -3 may have some adverse effects. In addition to the risk of allergic reactions to fish-derived ω -3 in individuals sensitive to fish, gastrointestinal disturbances, hepatic impairment, hyperglycemia, hypotension and bleeding are possible in excess doses [46]. Therefore, determining the exact dose and duration is essential to achieve the favored result.

Author contributions

SF led the study design, cytokine assay, data analysis and writing. MM led the study design, examined the patients, performed spirometry tests and writing. MK & TG assisted with cytokine assay and contributed in writing. SHT contributed substantially in statistical analyses and writing.

Conflict of interest

All authors declare no conflict of interest regarding our manuscript.

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