Original Article

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Immunomodulatory Effects of Omega-3 Fatty Acids in Patients with Differentiated Thyroid Cancer Before or After Radioiodine Ablation

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ABSTRACT

Background: Thyroid cancer and radioactive iodine (RAI) ablation for postsurgical management may lead to uncontrolled inflammation.

Objective: This study was intended to assess the prophylactic and therapeutic immunomodulatory effects of omega-3 fatty acids in patients with differentiated thyroid cancer (DTC).

Methods: A total of 85 patients with DTC were allocated into two groups based on RAI dosage after thyroidectomy. Patients in each group were randomly distributed into three subgroups: G1 with RAI ablation only, G2 treated with omega-3 for 30 days before RAI ablation, and G3 treated with omega-3 for 30 days after RAI ablation. Fifteen healthy individuals were included as controls. Serum cytokine levels including IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, TNF-α and IFN-γ were determined by cytometric bead assay.

Results: IL-4, IL-6, IL-21 and IL-22 levels in patients with DTC were higher than in the healthy controls. Regardless of RAI dosage, IL-6 showed an increasing trend after RAI ablation. IL-4, IL-22, and IL-17A remained at considerably higher levels than in the healthy controls after RAI ablation. Within-group comparisons showed a significant reduction in Th1+Th17/Th2+Th22 ratio in G2 patients 1 week after RAI ablation. Between-group comparisons showed increased IL-10 levels in G3 compared with G1 patients one week after high-dose RAI ablation. In G3, Th1+Th17/Th2+Th22 and Th1+Th17/Th2+Th9+Th22 ratios were remarkably lesser than in G2 patients 1 month after intermediate-dose RAI ablation.

Conclusion: Our results showed better anti-inflammatory effects of omega-3 when it was used therapeutically after RAI ablation in patients with DTC than when it was used prophylactically before RAI.

Keywords: Differentiated thyroid cancer, Radioiodine ablation, Omega-3, Cytokine

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INTRODUCTION

Thyroid cancer is the most prevalent endocrine malignancy, with an annual incidence of 4.6/100,000 and a female to male ratio of 3:1 in the USA (1). Based on the Iran Cancer Data System Registry, the average annual incidence rate of thyroid cancer was reported to be 2.2/100,000 between 2004 and 2010 (2). Thyroid cancers are classified into differentiated (papillary and follicular) and undifferentiated (anaplastic and medullary) carcinoma. About 90% of all thyroid cancers are differentiated thyroid carcinoma (DTC) (3, 4). Total or near-total thyroidectomy with lymph node dissection is the main treatment for DTC. Depending on the tumor stage and other criteria that can indicate a high risk of recurrence or disease-related mortality, subsequent radioactive iodine (^{131}I, RAI) ablation may also be recommended (5). Like normal thyroid follicular cells, malignant follicular cells accumulate radioiodine, which ablates cancer remnants and treats metastatic lesions. Although RAI ablation is very effective in the treatment of thyroid cancer, it is relatively toxic and has potential side effects (6).

Radioiodine-induced thyrocyte destruction and the release of large amounts of stress molecules such as damaged-associated molecules in addition to self-antigens may lead to uncontrolled inflammation and autoimmune thyroid disease. Although the induction of a specific immune response against malignant cells may favor cancer inhibition, increased inflammation can result in cancer progression (7, 8). Therefore, modulating excess inflammation in this condition may prevent possible side-effects.

Vitamins and dietary supplements have been studied as potential radioprotectors (9). Vitamins C and E are known antioxidants and free radical scavengers (10), and their radioprotective effects have been investigated previously (11). There is also a bulk of evidence supporting the beneficial effect of omega-3 fatty acids in patients with inflammatory diseases (12), autoimmune diseases (13), asthma (14), and cancers (15, 16).

Cytokines are bioactive molecules mainly produced by immune cells, especially helper T (Th) cells; however, tumor cells are also among various sources of cytokine secretion (17). Cytokines play a crucial role in the regulation of immune responses, and cytokine imbalances have been related to different diseases (18). Cytokine changes have been described in patients with different types of cancers (19, 20), but the data are limited regarding cytokine alterations in thyroid cancer and particularly after RAI ablation in patients with DTC (21-23).

Inflammation, recently considered as one of the hallmarks of cancer, plays an important role in the development and progression of thyroid cancers (24, 25). Because of the increasing incidence of DTC and the need for RAI ablation in the management of most patients, which in turn causes dysregulation of immune responses, and considering the role of omega-3 fatty acids in immune regulation, this study was intended to investigate the possible prophylactic or therapeutic immunomodulatory effects of omega-3 in patients with DTC before or after RAI ablation.

MATERIALS AND METHODS

Patients and Controls

The protocol of this study was approved by our University Ethics Committee (approval code IR.SUMS.REC.1398.1302) and registered at the Iranian Registry of Clinical Trials (IRCT code: IRCT20220104053622N1), 90 patients with DTC were included in this cross-sectional study from January 2018 to July 2020. All patients had a total or near-total thyroidectomy and were referred to the Nuclear Medicine Department at Namazi Hospital affiliated with Shiraz University of Medical Sciences for postsurgical RAI ablation.

The individual dosages were determined

by a nuclear medicine specialist based on the histological grade of each patient's tumor according to the American Thyroid Association Management Guidelines for Adult Patients with DTC (5). They were scheduled to receive postsurgical RAI ablation for 4 to 6 weeks after a 10-day low-iodine diet.

After obtaining written informed consent from participants, demographic data including age, sex, family history of thyroid diseases or any syndrome associated with DTC, smoking status, and physical exercise habits, which may affect cytokine profiles (26), were recorded. Clinicopathological data for each patient were also obtained from their medical records.

The patients were allocated into two groups based on RAI dosage: high-dose with 150 mCi (5.55 GBq) and intermediate-dose with 100 mCi (3.7 GBq). Then patients in each group were randomly distributed into three subgroups: G1 with RAI ablation only, G2 treated with omega-3 for 30 days before RAI ablation, and G3 treated with omega-3 for 30 days after RAI ablation.

Patients who received radioiodine at doses higher than 150 mCi, were admitted previously for RAI therapy before the study, had taken omega-3 or other supplements before the study, had undergone a second thyroid surgery, and had a concurrent chronic inflammatory disease, autoimmune disease, or other malignancies were excluded from the study.

Fifteen healthy individuals (sex- and age-matched with G1) with normal T3, T4, and TSH levels and negative findings for antithyroid antibodies were also included in this study as a control group.

Patients in G2 were instructed to take a soft-gel capsule of fish oil-derived omega-3 fatty acids (Best Formulations Inc., Los Angeles, CA, USA) containing 180 mg eicosapentaenoic acid and 120 mg docosahexaenoic acid daily for 30 consecutive days before RAI ablation, and those in G3 took the same capsule daily for 30 consecutive days after RAI ablation.

In G2, the first blood sample was collected 1 month before RAI ablation. Then patients started to take omega-3 for 30 days and the second blood sample was collected immediately before RAI ablation. The third and fourth blood samples were collected 1 week and 1 month after RAI ablation, respectively. In G1 and G3, blood samples were collected immediately before RAI ablation as well as 1 week and 1 month after RAI ablation. A blood sample was also collected from each individual in the control group. Serum was isolated from each blood sample and stored at −20 °C until cytokine assays, which were done in the same assay batch for all collected samples.

Cytokine Assay

The levels of 13 cytokines belonging to Th1 (IL-2, TNF-α, and IFN-γ), Th2 (IL-4, IL-5, IL-6, IL-10, and IL-13), Th17 (IL-17A, IL-17F, and IL-21), Th9 (IL-9), and Th22 (IL-22) families were measured with a commercial kit for multiplex cytometric bead assays (BioLegend, San Diego, CA, USA) based on the manufacturer's instructions. Briefly, after capturing the desired cytokines in serum samples or standards with a mixture of fluorescein isothiocyanate-labeled antibody-coated beads, differentiated by size and fluorescence intensity, a mixture of biotin-conjugated antibodies against each target cytokine and phycoerythrin-labeled streptavidin were added in succession. The results were analyzed with a FACSCalibur flow cytometer (eBioscience, San Diego, CA, USA) and the data were examined with FlowCytomix Pro-3.0 software (BioLegend). The minimum detectable concentration of each cytokine in serum with this kit was reported to be 0.9 pg/mL for IL-10, 0.9 pg/mL for TNF-α, 1.1 pg/mL for IL-4, IL-6 and IL-13, 1.2 pg/mL for IL-9, 1.3 pg/mL for IL-2, IL-5 and IL-17F, 1.4 pg/mL for IFN-γ, 1.8 pg/mL for IL-17A, 2.2 pg/mL for IL-22, and 2.3 pg/mL for IL-21.

Statistical Analysis

Cytokine levels were presented as the

mean ± standard error of the mean. Normal distribution of the data was evaluated with the Shapiro–Wilk test; because the data did not show a normal distribution, non-parametric statistical tests were used. Correlations between cytokine levels before any intervention with age, tumor size, and thyroglobulin serum level were calculated with Spearman's rank test, and correlations with sex and lymph node involvement were calculated with the Mann–Whitney U test. The distribution of qualitative demographic and clinicopathological variables among G1, G2, and G3 in each of the high-dose or intermediate-dose RAI ablation groups was examined separately with the chi-square test, and quantitative variables were evaluated with the Kruskal–Wallis test. The Wilcoxon test was used to compare cytokine levels at different time-points in each group. The Kruskal–Wallis test was used to compare cytokine levels among G1, G2, and G3 1 week and 1 month after RAI ablation with 100 mCi or 150 mCi. All statistical analyses were done with SPSS (SPSS version 22, SPSS, Inc., Chicago, USA), and a two-tailed P-value <0.05 was considered statistically significant. Bonferroni correction was applied for multiple comparison tests. GraphPad (GraphPad PRISM version 6, San Diego, USA) was used to create graphical displays.

RESULTS

A total of 90 patients with DTC have initially participated in the study. Five patients were excluded during the study: 3 because of non-compliance with the treatment and 2 because of a missed appointment for blood sampling. A total of 85 patients with papillary carcinoma completed the study: 11 males and 74 females, with a mean age of 37.5±11.9 years (range 18–67 years). All of these patients were non-smokers and none of them reported exercising regularly. None of them had a family history of thyroid cancer, and none had any other malignancies, active infections, chronic inflammatory or autoimmune diseases. The characteristics of patients in different subgroups are summarized in Table 1.

Correlation between demographic or

Table 1. Demographic and clinicopathological characteristics of patients with DTC scheduled for high-dose or intermediate-dose RAI ablation

Character-istics	High-dose RAI ablation (150 mCi) (n=43)			P-value	Intermediate-dose RAI ablation (100 mCi) (n=42)			P-value
	G1 (n=15)	G2 (n=15)	G3 (n=13)		G1 (n=15)	G2 (n=12)	G3 (n=15)	
Age (mean±SD)								
	35.00±10.92	39.60±10.48	34.23±11.49	0.365	39.73±13.08	37.50±12.90	38.87±13.56	0.904
Sex								
Male	4	4	1	0.373	1	0	1	0.657
Female	11	11	12		14	12	14	
Serum thyroglobulin level (ng/mL)								
<0.2	2	3	2	0.500	3	3	3	0.106
0.2-1	3	3	6		5	1	9	
>1-10	3	3	3		5	6	2	
>10	6	5	1		1	2	0	
Tumor size (cm)								
<1	6	8	8	0.246	10	10	10	0.300
1-2	6	2	3		5	1	5	
>2	1	4	1		0	1	0	
Lymph node involvement								
LN-	8	7	10	0.335	10	12	11	0.092
LN+	6	7	3		5	0	4	

clinicopathological factors and cytokine levels before any intervention in patients with DTC is shown in Table 2. Our results showed negative associations of age with IL-2, IL-17A, IL-17F levels and with all cytokine ratios, and positive associations of sex with IL-17F level before any intervention in patients scheduled for high-dose RAI ablation. We also found positive associations of tumor size with IL-9, IL-17A, TNF-α levels, and Th17/Th2 ratio before any intervention in patients scheduled for intermediate-dose RAI ablation.

Table 2. Correlation between demographic and clinicopathological factors and cytokine levels before any intervention in patients with DTC scheduled for RAI ablation

High-dose RAI ablation (150 mCi) (n=43)									
Cytokine	**Age**	**Tumor size**	**Tg**	**Sex (mean±SEM)**			**Lymph node involvement (mean±SEM)**		
	r (P-value)			**Male**	**Female**	**P-value**	**LN-**	**LN+**	**P-value**
IL-2	-0.312 (0.042)	-0.042 (0.798)	0.134 (0.409)	20.24±17.57	5.46±1.22	0.709	4.93±1.05	15.00±9.94	0.556
I-L4	-0.149 (0.340)	0.015 (0.929)	0.174 (0.284)	7.80±3.42	6.31±1.87	0.221	6.81±2.48	6.14±2.13	0.915
IL-5	-	-	-	0.65±0.00	0.65±0.00	1	0.65±0.00	0.65±0.00	1
IL-6	0.254 (0.100)	-0.037 (0.821)	0.217 (0.179)	5.06±1.28	6.80±2.01	0.893	7.24±2.68	4.62±0.85	0.968
IL-9 (Th9)	0.062 (0.694)	-0.197 (0.230)	0.170 (0.294)	0.60±0.00	0.71±0.06	0.362	0.75±0.08	0.60±0.00	0.155
IL-10	-0.113 (0.469)	-0.170 (0.302)	-0.052 (0.749)	2.41±0.49	3.48±0.59	0.521	3.42±0.76	2.61±0.42	0.947
IL-13	-0.047 (0.764)	-0.089 (0.591)	-0.120 (0.459)	4.46±1.39	3.54±0.72	0.317	3.74±0.96	3.92±0.85	0.43
IL-17A	-0.309 (0.044)	-0.055 (0.739)	-0.204 (0.207)	4.84±1.69	6.96±1.38	0.624	7.22±1.68	5.40±1.62	0.457
IL-17F	-0.377 (0.013)	0.080 (0.628)	-0.092 (0.571)	2.40±0.47	2.74±1.30	0.038	3.19±1.75	1.86±0.53	0.736
IL-21	-0.271 (0.078)	-0.165 (0.316)	0.137 (0.399)	16.63±5.83	7.49±1.73	0.136	8.08±2.33	8.91±2.79	0.785
IL-22 (Th22)	-0.258 (0.094)	-0.271 (0.095)	0.046 (0.777)	11.22±4.11	6.96±1.38	0.268	6.79±1.32	7.79±2.76	0.405
TNF-α	0.008 (0.962)	-0.092 (0.578)	0.185 (0.252)	2.16±0.71	1.94±0.47	0.539	1.77±0.52	1.72±0.53	0.935
IFN-γ	-0.193 (0.215)	-0.148 (0.370)	0.151 (0.354)	4.17±2.34	8.15±4.34	0.792	8.15±5.76	6.04±2.60	0.515
Th1	-0.216 (0.164)	-0.115 (0.485)	0.145 (0.372)	26.56±19.85	15.55±4.85	0.754	14.85±6.30	22.77±11.39	0.81
Th2	0.024 (0.880)	-0.019 (0.907)	0.079 (0.630)	20.39±5.63	20.78±4.99	0.612	21.86±6.64	17.94±3.73	0.894
Th17	-0.295 (0.055)	-0.081 (0.625)	-0.103 (0.528)	23.87±6.82	17.19±3.64	0.188	18.48±4.44	16.17±4.65	0.377
Th1/Th2	-0.323 (0.035)	-0.126 (0.446)	0.152 (0.350)	0.69±0.29	0.68±0.10	0.332	0.54±0.05	0.90±0.24	0.915
Th17/Th2	-0.457 (0.002)	-0.118 (0.474)	-0.176 (0.277)	1.24±0.33	0.85±0.12	0.42	0.90±0.13	0.89±0.22	0.181
Th1+Th17/Th2	-0.426 (0.004)	-0.126 (0.444)	0.078 (0.633)	1.93±0.41	1.53±0.17	0.403	1.44±0.15	1.79±0.34	0.968
Th1+Th17/ Th2+Th22	-0.358 (0.019)	-0.030 (0.856)	0.172 (0.289)	1.20±0.25	1.08±0.12	0.654	1.01±0.09	1.26±0.24	0.904

Cytokine	Age	Tumor size	Tg	Sex (mean±SEM)			Lymph node involvement (mean±SEM)		
Th1+Th17/ Th2+Th9+Th22	-0.347 (0.023)	-0.051 (0.758)	0.142 (0.382)	1.17±0.25	1.05±0.11	0.622	0.98±0.09	1.22±0.24	0.904
Intermediate-dose RAI ablation (100 mCi) (n=42)									
Cytokine	**Age**	**Tumor size**	**Tg**	**Sex (mean±SEM)**			**Lymph node involvement (mean±SEM)**		
	r (P-value)			**Male**	**Female**	**P-value**	**LN-**	**LN+**	**P-value**
IL-2	0.040 (0.802)	0.019 (0.907)	-0.084 (0.606)	1.15±0.42	5.75±2.44	0.174	6.38±2.95	2.39±0.64	0.263
IL-4	-0.030 (0.852)	0.157 (0.321)	0.042 (0.797)	1.99±1.08	7.71±1.66	0.345	6.43±1.53	11.10±4.92	0.5
IL-5	-	-	-	0.65±0.00	0.65±0.00	1	0.65±0.00	0.65±0.00	1
IL-6	-0.114 (0.472)	0.038 (0.810)	-0.273 (0.089)	2.57±1.82	8.57±1.78	0.249	7.32±1.57	11.80±5.59	0.915
IL-9 (Th9)	-0.031 (0.845)	0.106 (0.504)	0.143 (0.379)	0.60±0.00	7.51±6.78	0.6	8.98±8.21	0.60±0.00	0.22
IL-10	-0.086 (0.588)	0.244 (0.119)	-0.195 (0.227)	2.38±1.01	3.02±0.37	0.836	3.20±0.43	2.22±0.43	0.408
IL-13	0.095 (0.550)	0.062 (0.699)	0.041 (0.804)	3.89±2.32	3.38±0.46	0.744	3.24±0.46	4.02±1.24	0.689
IL-17A	-0.118 (0.456)	0.414 (0.006)	0.158 (0.330)	5.01±0.13	7.94±1.37	0.813	8.46±1.61	5.39±1.34	0.829
IL-17F	0.057 (0.721)	0.229 (0.145)	-0.016 (0.922)	0.79±0.03	3.28±0.79	0.495	2.49±0.49	5.62±3.06	0.235
IL-21	0.048 (0.761)	0.376 (0.014)	-0.032 (0.846)	1.81±0.66	16.21±4.36	0.42	12.41±2.87	26.91±16.64	0.311
IL-22 (Th22)	0.091 (0.568)	0.216 (0.169)	-0.100 (0.539)	8.75±3.39	8.74±1.54	0.636	8.19±1.74	10.73±2.60	0.176
TNF-α	-0.005 (0.975)	0.319 (0.040)	0.192 (0.236)	0.45±0.00	2.72±0.62	0.279	2.92±0.72	1.48±0.70	0.301
IFN-γ	0.011 (0.944)	0.148 (0.351)	-0.235 (0.144)	7.85±7.15	14.28±3.65	0.922	13.37±3.88	16.17±8.32	0.786
Th1	0.044 (0.783)	0.119 (0.452)	-0.134 (0.410)	9.45±7.57	22.75±4.90	0.595	22.68±5.54	20.05±8.72	0.976
Th2	-0.106 (0.505)	0.178 (0.259)	-0.060 (0.714)	11.48±6.22	23.32±3.74	0.478	20.84±3.39	29.80±11.48	0.724
Th17	0.053 (0.739)	0.333 (0.031)	-0.014 (0.932)	7.60±0.82	27.43±5.62	0.768	23.36±4.60	37.93±19.06	0.276
Th1/Th2	0.100 (0.530)	0.095 (0.548)	-0.082 (0.613)	0.66±0.30	0.89±0.15	0.859	0.96±0.17	0.58±0.08	0.83
Th17/Th2	0.150 (0.342)	0.322 (0.038)	0.068 (0.675)	0.99±0.61	1.17±0.16	1	1.08±0.17	1.43±0.35	0.25
Th1+Th17/Th2	0.146 (0.357)	0.233 (0.138)	0.045 (0.783)	1.65±0.31	2.06±0.26	0.813	2.04±0.30	2.01±0.35	0.399
Th1+Th17/ Th2+Th22	0.147 (0.352)	0.170 (0.281)	0.029 (0.860)	0.81±0.22	1.44±0.18	0.555	1.46±0.21	1.23±0.17	0.702
Th1+Th17/ Th2+Th9+Th22	0.149 (0.347)	0.196 (0.214)	0.026 (0.873)	0.79±0.22	1.37±0.17	0.637	1.38±0.21	1.20±0.17	0.592

Tg: Thyroglobulin

As shown in Figure 1, the comparison of G1 patients (who received regular treatment) with the healthy controls revealed increased levels of IL-6, IL-4 and consequently Th2, as well as IL-21 and IL-22 in patients with DTC. Moreover, IL-2 as well as IL-17A and IL-21, and consequently Th17 also elevated in patients scheduled for RAI ablation with an intermediate dose. Regardless of the ^{131}I dosage, IL-6 showed an increasing trend after RAI ablation. IL-4, IL-22, and IL-17A remained at significantly higher levels after RAI ablation than the healthy controls.

Comparisons of cytokine levels at different time-points in patients with DTC who had RAI ablation with or without omega-3 treatment are illustrated in Figure 2. In G1 patients, IL-10 level significantly increased (P=0.01) 1 month compared with 1 week after high-dose RAI ablation, and IL-17F and IL-21 levels decreased (P=0.008 and P=0.041, respectively) 1 week after intermediate-dose RAI ablation compared with the time-point immediately before RAI ablation. There were no differences in Th subset cytokines or ratios between the two time-points (Supplementary Table 1).

In G2 patients (pretreated with omega-3), despite the absence of changes in the level of any of the cytokines or Th subset cytokines, Th1+Th17/Th2+Th22 ratio significantly decreased (P=0.029) 1 week after high-dose RAI ablation compared with just before ablation. In G2 patients with intermediate-dose RAI, IL-4 was significantly lower (P=0.001) 1 month after ablation compared with the time-point immediately before ablation. IL-13 showed a significant but transient increase (P=0.042) after pretreatment with omega-3 which returned to the baseline level after 1 month, whereas Th1+Th17/Th2+Th22 and Th1+Th17/Th2+Th9+Th22 ratios significantly decreased (P=0.042 for both) 1 week after RAI ablation compared with just before ablation (Supplementary Table 2).

In G3 patients who received high-dose RAI and post-treatment with omega-3, IL-6 (P=0.033), IL-13 (P=0.032), and Th2 cytokines (P=0.027) significantly increased

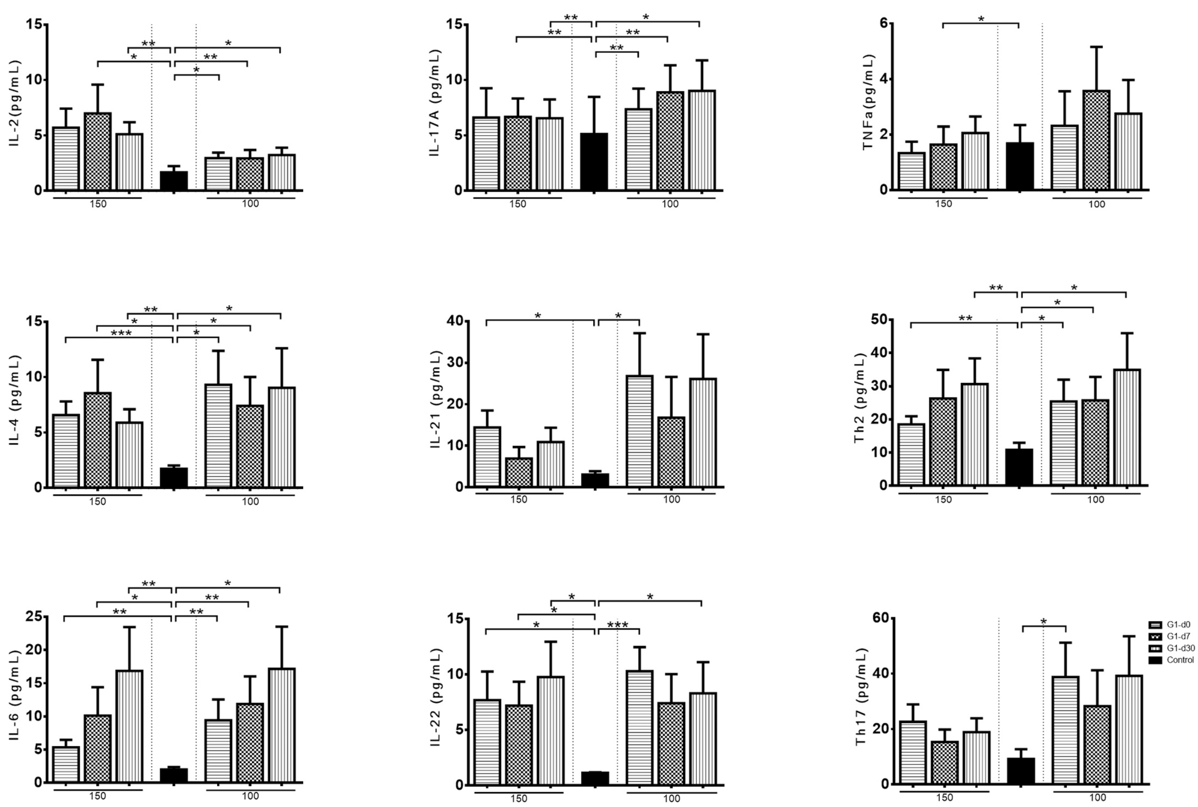

Figure 1. Cytokine changes in patients with DTC before and after RAI ablation compared with the healthy controls (* indicates P<0.05, ** indicates P<0.01).

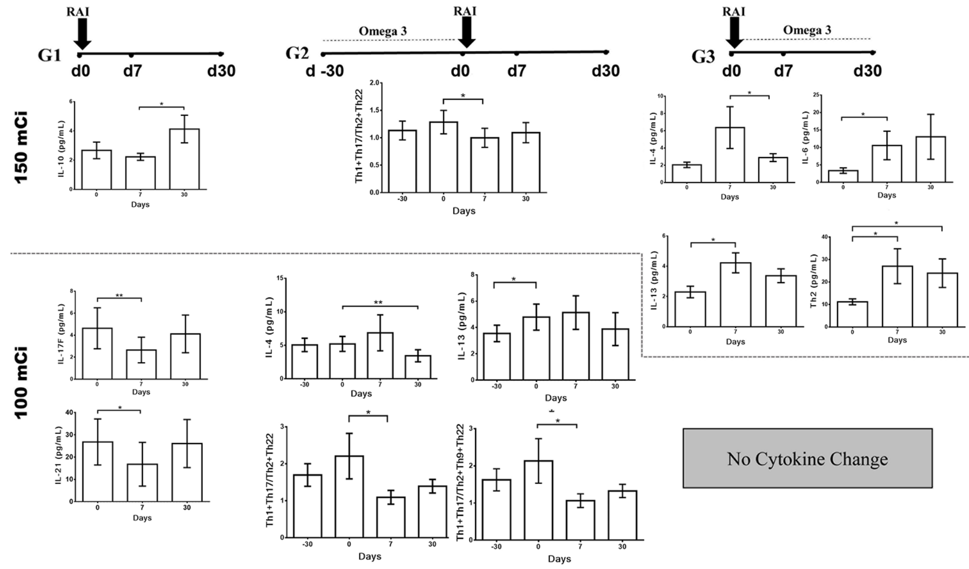

Figure 2. Cytokine changes in patients with DTC at different time points after RAI ablation preceded or followed by omega-3 fatty acid treatment (* indicates P<0.05, ** indicates P<0.01).

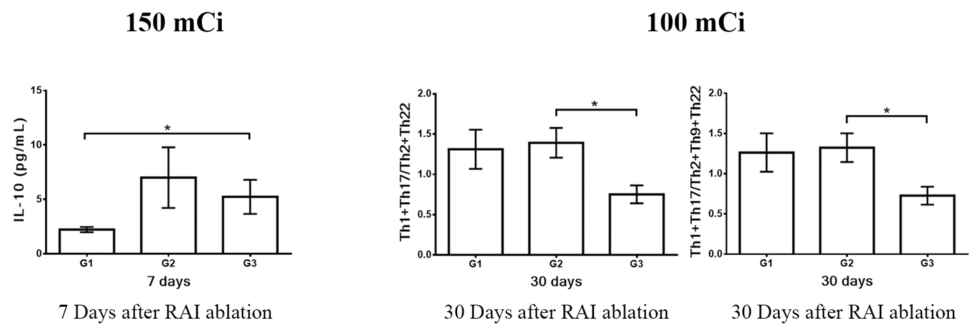

Figure 3. Between-group comparisons of cytokines in patients with DTC 1 week and 1 month after RAI ablation: G1 with RAI ablation only, G2 treated with omega-3 fatty acids for 30 days before RAI ablation, and G3 treated with omega-3 for 30 days after RAI ablation (* indicates P<0.05).

1 week after treatment. The significant increase in Th2 was maintained for up to 1 month after treatment (P=0.027); however, IL-4 level decreased significantly (P=0.042) 1 month compared with 1 week after the intervention. Post-treatment with omega-3 in patients who received intermediate-dose RAI was not related with any remarkable changes in any of the cytokines tested, or in Th subset cytokines or ratios (Supplementary Table 3).

Between-group comparisons of cytokine levels are summarized in Figure 3. As shown, IL-10 levels in G2, and G3 were higher than in G1 patients 1 week after high-dose RAI ablation (pairwise comparison: G2 vs. G1, p=0.038 and G3 vs. G1, p=0.013), although only the difference between G3 and G1 remained significant after Bonferroni adjustment.

Significantly lower Th1+Th17/Th2+Th22 (P=0.007) and Th1+Th17/Th2+Th9+Th22 ratios (P=0.01) were seen in G3 compared with G2 patients 1 month after intermediate-dose RAI ablation, although these ratios were similar in G1 and G2.

DISCUSSION

The increasing incidence of thyroid cancer requires intensive efforts to improve the early diagnosis and management of these patients. Cancer outcomes are strongly influenced by the cytokine network, which profoundly affects immune responses. It is well established that Th1 cytokines support antitumor immune responses whereas Th2 cytokines facilitate tumor progression due to the downmodulation of cell-mediated immunity (27). In addition, some data show that inflammation is a critical component in tumor progression. In consonance with these earlier findings, our data showed higher IL-6 levels in patients with DTC compared with the healthy controls (Figure 1). A strong link between DTC and inflammation has also been reported (28) and was attributed to inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-17F produced not only by tumor infiltrating leukocytes but also by tumor cells themselves (29). We also observed elevated IL-22 levels in DTC patients compared with the normal controls. IL-22 has both pro-inflammatory and anti-inflammatory effects and reportedly plays a role in the migration and invasion of papillary thyroid cancer cells (30). Higher Th2 and especially IL-4 levels in patients with DTC versus healthy controls in our study may promote tumor progression and tumor resistance to chemotherapy by upregulating anti-apoptotic proteins (31). Elevated IL-2 and increased Th17 due to increased IL-17A and IL-21 production were observed only in DTC patients scheduled for intermediate-dose RAI ablation in comparison with the healthy controls (Figure 1). Although IL-2 and IL-17A can induce HLA class I overexpression in malignant cells, which makes them better targets for cytotoxic T cells, elevated IL-17A production suppresses cytotoxic T cells and eventually promotes metastases (32, 33).

Despite the direct or inverse correlations of some cytokines with age, sex, or tumor size (Table 2), patients in the present study were almost uniformly distributed among G1, G2, and G3 in each of the two main groups scheduled for high-dose or intermediate-dose RAI ablation, and none of the demographic or clinicopathological features appeared to have a confounding effect on cytokine levels (Table 1).

Therapeutic procedures including radiotherapy affect cytokine levels, which in turn may affect immune responses against malignant cells (19, 34, 35). In this connection, we noted an increasing trend in IL-6 among patients with DTC after RAI ablation (G1). Moreover, IL-17A, IL-22, and IL-4 levels in these patients remained higher than in the normal controls even after RAI ablation. Jones et al. reported transient increases in IL-4, IL-6, and IL-10 after RAI therapy for Graves' disease (34). Ozata et al. also found a significant increase in IL-6 2 months after RAI therapy in patients with DTC, in the absence of changes in TNF-α levels (21). Georgakilas et al. (36) and Yahyapour et al. (37) provided some evidence for radiation-induced inflammation. The results of our study disclosed increased levels of IL-10 1 month after high-dose RAI ablation. Although increased IL-10 downmodulates immune responses against malignant cells, it may also prevent metastases by reducing inflammation. Our results, however, are inconsistent with the findings reported by Demir et al., who observed some cellular evidence of increased systemic inflammation 2 months after RAI ablation with 100 or 150 mCi in patients with DTC (38). We also observed significant reductions in IL-17F and IL-21 levels 1 week after intermediate-dose RAI ablation in our patients (Figure 2, left). Although IL-17F and IL-21 are both pro-inflammatory cytokines (39) produced mainly by Th17 cells, we found no significant decrease in overall Th17-related cytokines – a result that can be explained by the non-significant increase in IL-17A. Decreases in IL-17F and IL-21 after RAI ablation may prevent tumor invasion by reducing inflammation. Our results are inconsistent with the findings published by Zhang et al., who reported an elevated frequency of Th17 cells and their related serum cytokines in peripheral blood from patients with DTC compared with the healthy controls, a significant decline in these markers 1 week after ^{131}I therapy, and a return to normal levels after 3 months (23). It is worth noting that, considering the complex interactions among cytokines within an extensive network, increased IL-10 alone or decreased IL-17 and IL-21 with no effect on overall cytokine balance may not result in any specific biological effect (40).

There is evidence in support of the beneficial effects of omega-3 on recovery in patients who undergo chemotherapy, radiotherapy, or both (41). Selective cytotoxicity of omega-3 against cancer cells was reported by D'Eliseo et al., who suggested prescribing omega-3 in combination with conventional anticancer therapies (42). To discover whether omega-3 had any beneficial prophylactic effect in patients treated with RAI ablation, a group of our patients with DTC was pretreated with omega-3 for 30 days (G2). Although we found no change in the level of any cytokines or Th subset cytokines, Th1+Th17/Th2+Th22 ratio significantly decreased 1 week after high-dose RAI ablation in G2 patients, which indicated an overall decline in inflammatory cytokines or an increase in anti-inflammatory cytokines. Although our findings should be considered preliminary, these changes may curtail high-grade tumor progression. In patients who were pretreated with omega-3 and then received intermediate-dose RAI, IL-13 increased immediately after the course of omega-3, whereas IL-4 substantially decreased 1 month after RAI ablation. Despite the non-significant increase in Th2, Th1+Th17/Th2+Th22 and Th1+Th17/Th2+Th9+Th22 ratios after omega-3 consumption, these ratios decreased 1 week after RAI ablation (Figure 2, middle). This might be explained by the decrease in the sum of minor changes in Th1- and/or Th17-related cytokines, or by the increase in the sum of minor changes in Th9-, Th22- or Th2-related cytokines. Our results showed that omega-3 prophylaxis in patients with DTC shifted the overall cytokine balance towards a temporary increase in cellular immunity immediately before RAI ablation, which may be critical for the effective removal of malignant cells, and then redirection of the overall cytokine balance toward a reduction in inflammation 1 week after RAI ablation, which may have been helpful in preventing metastases in our patients who received high-dose or intermediate-dose RAI ablation.

To investigate the therapeutic effects of omega-3 in patients treated with RAI ablation, a group of our patients with DTC was post-treated with omega-3 for 30 days after ablation (G3). Our results showed significant elevations in IL-4, IL-6, IL-13, and consequently Th2 cytokine levels 1 week after high-dose RAI ablation. Despite successive decreases in IL-4 and IL-13 levels after 1 month, Th2 elevation was maintained for up to 1 month, which can be explained by the increasing trend in IL-6 1 month after treatment (Figure 2, right), even though Th2 elevation had no remarkable effect on cytokine ratios. Although Th2 activity prevents Th1-mediated antitumor responses, IL-6, a member of the Th2 cytokines, is also produced by other cells and is a pro-inflammatory cytokine which can lead to extensive tumor expansion (43). In contrast to our finding of increased Th2 cytokine levels 1 week after RAI ablation with 150 mCi in combination with omega-3, Simonovic et al. reported reduced Th2 cytokines in the supernatant of phytohemagglutinin-stimulated peripheral blood cells of patients with DTC 1 week after treatment with 100 mCi RAI (22). This inconsistency may be viewed as an effect of omega-3 in the present study. It may also be due to the different doses of RAI used in these two studies, or the explanation may lie in more complex interactions among various factors in the present *in vivo* study compared with the *in vitro* study by Simonovic et al.

Despite the elevation in IL-10 in G2 and G3 compared with G1 1 week after high-dose RAI ablation, which is possibly owing to omega-3 consumption, a significant difference was observed only between G3 and G1 (Figure 3). Although the lack of significant difference between G2 and G1 may be explained by internal variation in IL-10 levels among G2 patients, it can still be clinically important.

In response to the question of whether the increase in IL-10 is the result of RAI ablation or omega-3 consumption, due to the lack of significant change in this cytokine 1 week and 1 month after RAI ablation compared with the baseline in G1 patients, the increase in IL-10 in G2 and G3 can be the result of omega-3 consumption.

The transient reduction in IL-17F and IL-21 in G1 patients 1 week after intermediate-dose RAI ablation can also be considered a favorable effect of RAI, although both returned to baseline values after 1 month. Although these two cytokines showed no significant changes in G2 or G3, Th1+Th17/Th2+Th22 and Th1+Th17/Th2+Th9+Th22 ratios were significantly lower in G3 compared with G2 (Figure 3).

Overall, our results document better anti-inflammatory effects of omega-3 when it was used therapeutically after RAI ablation in patients with DTC than when it was used prophylactically before RAI ablation.

The main strengths of this study were the inclusion of a large number of patients with DTC who received different doses of RAI, and the simultaneous evaluation, with a precise quantitative method, of 13 cytokines belonging to the main Th subsets. One limitation of our study was the short-term follow-up; longer follow-up periods are needed to evaluate the efficacy of omega-3 in enhancing the immune profile and improving the prognosis in patients with cancer. All patients in the present study received the same dose of omega-3 for a period of 30 days before or after RAI ablation. Long-term studies of omega-3 consumption starting some time before radiotherapy and continuing after therapy, with different doses of omega-3 for different durations, may shed additional light on the potential immune regulatory benefits of this dietary supplement. Another limitation of this study was the lack of a placebo-controlled group. Olive oil has previously been applied as a placebo in similar studies, but because of its potentially immunomodulatory effects, it may confound the results (44, 45). To clarify the points that remain obscure, future analyses could focus on intracellular cytokine production by different T cell subsets in peripheral blood, tumor-infiltrating T cells, tumor-draining lymph nodes, and tumor cells in patients with DTC who receive a combination of RAI and omega-3.

CONCLUSION

Our results showed better anti-inflammatory effects of omega-3 when it was used therapeutically after RAI ablation in DTC patients than when it was used prophylactically before RAI ablation.

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Conflicts of Interest: None declared.

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Table S1. Cytokine changes at different time points after RAI ablation in patients with DTC (G1)

Cytokine	Sampling time points	High-dose RAI ablation (150 mCi) n=15				Intermediate-dose RAI ablation (100 mCi) n=15			
		Mean±SEM	P-value			Mean±SEM	P-value		
			d0 vs. d7	d0 vs. d30	d7 vs. d30		d0 vs. d7	d0 vs. d30	d7 vs. d30
IL-2	d0	5.70±1.72	0.639	0.934	0.703	2.95±0.50	0.679	0.761	0.890
	d7	6.99±2.59				2.93±0.76			
	d30	5.10±1.09				3.23±0.67			
IL-4	d0	6.57±1.24	0.330	0.561	0.489	9.31±3.06	0.151	0.208	0.599
	d7	8.55±3.01				7.41±2.60			
	d30	5.89±1.20				9.02±3.58			
IL-5	d0	0.65±0.00	1.000	1.000	1.000	0.65±0.00	1.000	1.000	1.000
	d7	0.98±0.33				0.65±0.00			
	d30	0.74±0.09				0.65±0.00			
IL-6	d0	5.35±1.12	0.808	0.135	0.421	9.43±3.12	0.978	0.277	0.599
	d7	10.13±4.27				11.87±4.14			
	d30	16.85±6.60				17.16±6.35			
IL-9 (Th9)	d0	0.75±0.10	0.500	1.000	0.500	0.66±0.06	0.500	0.500	0.875
	d7	1.11±0.37				0.80±0.14			
	d30	0.71±0.11				0.93±0.26			
IL-10	d0	2.67±0.57	0.847	0.057	0.010	2.81±0.71	0.599	0.524	0.561
	d7	2.23±0.24				2.78±0.50			
	d30	4.13±0.94				3.05±0.88			
IL-13	d0	3.25±0.50	0.639	0.303	0.268	3.21±0.79	0.946	0.839	0.340
	d7	4.38±1.28				3.01±0.81			
	d30	3.01±0.61				5.02±1.33			
IL-17A	d0	6.62±2.64	0.470	0.454	0.879	7.36±1.86	0.978	0.967	0.910
	d7	6.67±1.66				8.88±2.45			
	d30	6.55±1.69				9.02±2.76			
IL-17F	d0	1.60±0.31	1.000	0.622	0.569	4.63±1.86	0.008	0.073	0.787
	d7	1.70±0.35				2.65±1.16			
	d30	1.42±0.24				4.12±1.71			
IL-21	d0	14.41±4.09	0.064	0.519	0.193	26.78±10.32	0.041	1.000	0.147
	d7	6.96±2.73				16.76±9.81			
	d30	10.91±3.46				26.07±10.77			
IL-22 (Th22)	d0	7.69±2.57	0.765	0.359	0.421	10.29±2.17	0.463	0.326	0.853
	d7	7.19±2.15				7.42±2.61			
	d30	9.76±3.19				8.30±2.81			
TNF-α	d0	1.33±0.41	1.000	0.148	0.297	2.32±1.24	0.338	0.562	0.848
	d7	1.64±0.65				3.57±1.59			
	d30	2.06±0.59				2.75±1.22			
IFN-γ	d0	5.44±2.58	0.312	0.437	0.625	15.24±6.65	0.301	0.742	1.000
	d7	3.85±1.55				10.47±5.15			
	d30	16.32±14.90				26.42±16.75			
Th1	d0	12.47±3.47	0.421	0.804	0.670	20.51±7.62	0.639	0.454	0.188
	d7	12.47±3.84				16.97±6.46			
	d30	23.48±14.88				32.39±18.22			
Th2	d0	18.49±2.47	0.679	0.151	0.359	25.41±6.56	0.629	0.847	0.847
	d7	26.28±8.62				25.72±7.06			
	d30	30.62±7.75				34.91±11.03			

Th17	d0	22.63±6.29	0.241	0.847	0.669	38.76±12.41	0.107	0.890	0.376
	d7	15.33±4.49				28.29±12.95			
	d30	18.88±5.00				39.21±14.28			
Th1/Th2	d0	0.57±0.12	0.429	0.169	0.501	0.66±0.19	0.626	0.296	0.599
	d7	0.49±0.10				0.68±0.19			
	d30	1.16±0.81				0.64±0.21			
Th17/Th2	d0	1.09±0.23	0.135	0.599	0.639	1.47±0.28	0.147	0.389	0.890
	d7	0.79±0.22				1.00±0.24			
	d30	0.85±0.21				1.13±0.28			
Th1+Th17/Th2	d0	1.66±0.30	0.151	0.720	0.934	2.13±0.42	0.359	0.359	0.978
	d7	1.29±0.28				1.68±0.39			
	d30	2.01±0.87				1.77±0.39			
Th1+Th17/ Th2+Th22	d0	1.14±0.16	0.188	0.421	0.847	1.45±0.31	0.277	0.561	0.804
	d7	0.85±0.12				1.25±0.24			
	d30	1.46±0.65				1.31±0.24			
Th1+Th17/ Th2+Th9+Th22	d0	1.10±0.16	0.173	0.454	0.772	1.41±0.30	0.277	0.524	0.847
	d7	0.82±0.12				1.20±0.23			
	d30	1.42±0.64				1.26±0.24			

Table S2. Cytokine changes at different time points in patients with DTC pretreated with omega-3 for 30 days before RAI ablation (G2)

Cytokine	Sampling time points	High-dose RAI ablation (150 mCi) (n=15)						
		Mean±SEM	P-value					
			d30 vs. d0	d30 vs. d7	d30 vs. d30	d0 vs. d7	d0 vs. d30	d7 vs. d30
IL-2	d-30	16.59±10.53	0.808	0.489	0.761	0.847	0.107	0.847
	d0	12.54±5.84						
	d7	11.13±4.69						
	d30	9.85±4.65						
IL-4	d-30	10.63±4.32	0.890	0.454	0.761	0.934	0.893	0.978
	d0	8.27±2.89						
	d7	7.94±2.30						
	d30	6.79±1.72						
IL-5	d-30	0.65±0.00	1.000	1.000	1.000	1.000	1.000	1.000
	d0	0.65±0.00						
	d7	0.65±0.00						
	d30	0.65±0.00						
IL-6	d-30	10.19±4.34	0.599	0.330	0.891	0.804	0.188	0.330
	d0	23.28±9.21						
	d7	559.45±539.01						
	d30	7.38±1.64						
IL-9 (Th9)	d-30	0.70±0.10	1.000	0.500	1.000	0.750	1.000	0.750
	d0	0.75±0.15						
	d7	1.04±0.33						
	d30	0.74±0.14						
IL-10	d-30	4.22±1.16	0.761	0.670	0.934	0.804	0.855	0.639
	d0	4.43±0.97						
	d7	7.00±2.79						
	d30	3.99±0.62						

IL-13	d-30	5.46±1.67	0.793	0.903	0.709	0.283	0.847	0.649
	d0	5.42±1.42						
	d7	5.83±1.31						
	d30	5.08±0.97						
IL-17A	d-30	6.31±1.50	0.670	0.194	1.000	0.340	0.470	0.233
	d0	8.12±1.74						
	d7	11.21±2.92						
	d30	6.30±1.92						
IL-17F	d-30	4.98±2.90	0.183	1.000	0.382	0.465	0.985	0.658
	d0	3.42±1.39						
	d7	5.11±1.89						
	d30	2.97±1.13						
IL-21	d-30	9.52±2.90	0.986	0.275	0.970	0.339	0.831	0.366
	d0	7.65±2.00						
	d7	17.12±6.88						
	d30	6.88±2.14						
IL-22 (Th22)	d-30	8.34±2.46	0.730	0.296	0.893	0.229	1.000	0.454
	d0	7.60±2.07						
	d7	45.08±29.90						
	d30	7.39±1.87						
TNF-α	d-30	3.30±0.90	0.557	0.250	0.250	0.250	1.000	0.164
	d0	3.00±1.10						
	d7	8.70±4.39						
	d30	2.18±0.70						
IFN-γ	d-30	12.56±9.60	0.328	0.562	0.461	0.734	0.465	0.898
	d0	24.42±11.71						
	d7	17.43±7.19						
	d30	8.65±2.27						
Th1	d-30	32.46±15.03	0.561	0.639	0.804	0.934	0.359	0.890
	d0	39.96±13.78						
	d7	37.26±11.01						
	d30	20.68±5.66						
Th2	d-30	31.14±11.07	0.679	0.761	0.761	0.890	0.277	0.524
	d0	42.06±11.60						
	d7	580.86±538.66						
	d30	23.89±4.27						
Th17	d-30	20.81±6.22	0.730	0.524	0.847	0.389	0.761	0.359
	d0	19.19±4.47						
	d7	33.44±9.93						
	d30	16.15±4.29						
Th1/Th2	d-30	0.82±0.23	0.252	0.421	0.934	0.421	0.367	0.421
	d0	1.02±0.25						
	d7	0.77±0.21						
	d30	0.84±0.17						
Th17/Th2	d-30	0.74±0.16	0.945	0.945	0.890	0.689	0.599	0.815
	d0	0.73±0.21						
	d7	0.73±0.16						
	d30	0.73±0.21						
Th1+Th17/Th2	d-30	1.55±0.25	0.270	0.720	0.720	0.583	0.426	0.804
	d0	1.74±0.34						
	d7	1.50±0.32						
	d30	1.57±0.32						

Th1+Th17/ Th2+Th22	d-30	1.13±0.17	0.192	0.381	0.599	0.029	0.229	0.808
	d0	1.29±0.21						
	d7	1.00±0.17						
	d30	1.09±0.18						
Th1+Th17/ Th2+Th9+Th22	d-30	1.10±0.17	0.147	0.389	0.524	0.055	0.213	0.761
	d0	1.25±0.21						
	d7	0.97±0.17						
	d30	1.06±0.18						
Intermediate-dose RAI ablation (100 mCi) (n=12)								
IL-2	d-30	11.25±8.08	0.470	0.622	0.176	0.733	0.301	0.733
	d0	12.08±8.53						
	d7	14.70±9.37						
	d30	6.01±3.35						
IL-4	d-30	5.06±1.00	0.765	0.831	0.102	0.301	0.001	0.233
	d0	5.21±1.12						
	d7	6.86±2.69						
	d30	3.45±0.90						
IL-5	d-30	0.65±0.00	1.000	1.000	1.000	1.000	1.000	1.000
	d0	0.65±0.00						
	d7	0.65±0.00						
	d30	0.65±0.00						
IL-6	d-30	6.42±3.07	0.622	0.240	0.733	0.910	1.000	0.470
	d0	5.84±1.95						
	d7	24.85±15.76						
	d30	5.60±1.85						
IL-9 (Th9)	d-30	0.97±0.20	0.750	0.500	0.750	0.500	1.000	1.000
	d0	0.99±0.26						
	d7	0.86±0.19						
	d30	0.87±0.27						
IL-10	d-30	3.21±0.70	0.569	0.791	0.677	0.519	0.733	0.850
	d0	4.38±1.13						
	d7	4.20±1.07						
	d30	3.48±0.98						
IL-13	d-30	3.55±0.62	0.042	0.700	0.638	0.791	0.204	0.677
	d0	4.79±0.99						
	d7	5.14±1.28						
	d30	3.88±1.25						
IL-17A	d-30	10.87±3.21	0.733	0.765	0.320	0.204	0.301	0.765
	d0	10.96±1.94						
	d7	9.44±2.90						
	d30	7.76±2.99						
IL-17F	d-30	2.17±0.61	0.233	0.850	1.000	0.519	0.123	0.831
	d0	2.91±0.63						
	d7	2.59±0.73						
	d30	1.92±0.39						
IL-21	d-30	14.00±5.23	0.820	0.426	0.203	0.966	0.164	1.000
	d0	11.88±3.61						
	d7	10.75±3.42						
	d30	7.71±2.21						
IL-22 (Th22)	d-30	9.79±3.61	0.733	0.910	0.240	0.791	0.151	0.193
	d0	9.19±2.16						
	d7	7.95±2.08						
	d30	4.92±1.26						

TNF-α	d-30	3.70±0.81	0.322	0.700	0.492	0.937	1.000	0.344
	d0	3.00±1.12						
	d7	4.06±1.41						
	d30	2.73±0.83						
IFN-γ	d-30	12.93±6.60	0.244	0.578	0.461	0.195	0.081	0.687
	d0	27.65±10.15						
	d7	7.35±3.66						
	d30	5.19±2.09						
Th1	d-30	27.88±11.64	0.380	0.424	0.301	0.301	0.110	0.910
	d0	42.73±15.68						
	d7	26.11±11.89						
	d30	13.92±4.02						
Th2	d-30	18.90±4.60	0.424	0.519	0.266	0.791	0.339	0.424
	d0	20.87±3.87						
	d7	41.70±16.04						
	d30	17.05±4.49						
Th17	d-30	27.04±8.68	0.677	0.380	0.380	0.424	0.176	0.850
	d0	25.75±5.17						
	d7	22.78±6.36						
	d30	17.39±4.60						
Th1/Th2	d-30	1.09±0.25	0.677	0.339	0.470	0.151	0.380	0.970
	d0	1.65±0.50						
	d7	0.80±0.23						
	d30	0.79±0.14						
Th17/Th2	d-30	1.34±0.30	0.970	0.147	0.233	0.110	0.176	0.518
	d0	1.40±0.30						
	d7	1.05±0.29						
	d30	1.05±0.22						
Th1+Th17/Th2	d-30	2.43±0.41	0.519	0.233	0.233	0.129	0.092	0.733
	d0	3.04±0.71						
	d7	1.85±0.48						
	d30	1.85±0.25						
Th1+Th17/ Th2+Th22	d-30	1.70±0.30	0.622	0.064	0.233	0.042	0.092	0.151
	d0	2.21±0.62						
	d7	1.09±0.19						
	d30	1.39±0.18						
Th1+Th17/ Th2+Th9+Th22	d-30	1.62±0.30	0.622	0.077	0.266	0.042	0.092	0.176
	d0	2.13±0.60						
	d7	1.06±0.18						
	d30	1.32±0.18						

Table S3. Cytokine changes in patients with DTC at different time points after RAI ablation and post-treatment with omega-3 for 30 days (G3)

Cytokine	Sampling time points	High-dose RAI ablation (150 mCi) (n=13)				Intermediate-dose RAI ablation (100 mCi) (n=15)			
		Mean±SEM	P-value			Mean±SEM	P-value		
			d0 vs. d7	d0 vs. d30	d7 vs. d30		d0 vs. d7	d0 vs. d30	d7 vs. d30
IL-2	d0	2.57±0.32	0.110	0.635	0.497	3.53±0.77	0.599	0.966	0.715
	d7	5.05±1.73				5.49±2.16			
	d30	3.09±0.45				3.45±0.73			
IL-4	d0	2.05±0.31	0.077	0.305	0.042	7.45±3.19	0.720	0.524	0.720
	d7	6.37±2.42				4.24±1.31			
	d30	2.89±0.45				7.19±3.14			
IL-5	d0	0.65±0.00	1.000	1.000	1.000	0.65±0.00	1.000	1.000	1.000
	d7	0.65±0.00				0.79±0.14			
	d30	0.65±0.00				0.65±0.00			
IL-6	d0	3.37±0.78	0.033	0.376	0.910	8.61±2.80	0.901	0.359	0.679
	d7	10.57±4.07				7.16±1.79			
	d30	13.05±6.42				15.29±6.18			
IL-9 (Th9)	d0	0.60±0.00	1.000	1.000	1.000	18.68±18.08	1.000	1.000	1.000
	d7	0.79±0.19				7.67±7.07			
	d30	0.60±0.00				2.12±1.52			
IL-10	d0	2.84±0.55	0.151	0.146	0.542	2.98±0.43	0.421	0.761	0.639
	d7	5.23±1.56				3.40±0.46			
	d30	3.99±0.88				3.11±0.53			
IL-13	d0	2.30±0.38	0.032	0.064	0.266	3.49±0.86	0.945	0.787	0.489
	d7	4.22±0.66				3.85±0.85			
	d30	3.37±0.46				3.66±0.95			
IL-17A	d0	6.64±1.65	0.638	1.000	0.569	5.80±1.80	0.519	0.966	1.000
	d7	11.50±4.03				7.37±2.89			
	d30	6.45±1.65				5.82±1.47			
IL-17F	d0	1.24±0.26	0.569	0.582	0.497	2.48±0.89	0.700	0.960	0.960
	d7	2.89±0.94				1.57±0.33			
	d30	1.61±0.28				1.91±0.68			
IL-21	d0	3.49±1.37	0.105	0.820	0.375	5.48±1.77	1.000	0.937	0.937
	d7	8.49±3.01				5.90±2.48			
	d30	4.45±1.60				6.10±2.17			
IL-22 (Th22)	d0	7.47±2.31	0.127	0.588	0.223	6.35±2.04	1.000	0.594	0.893
	d7	12.32±3.18				6.81±2.21			
	d30	8.49±1.88				6.60±1.51			
TNF-α	d0	1.23±0.45	0.148	0.312	0.383	2.04±0.90	0.406	1.000	0.437
	d7	4.47±1.93				2.99±1.39			
	d30	2.83±1.08				1.77±0.47			
IFN-γ	d0	3.42±1.36	0.055	0.297	0.312	13.53±5.29	0.219	0.742	0.641
	d7	18.72±10.21				7.10±2.48			
	d30	6.66±2.22				12.40±8.26			
Th1	d0	7.21±1.82	0.127	0.376	0.376	19.10±5.89	0.599	0.804	0.542
	d7	28.24±13.66				15.59±4.26			
	d30	12.59±3.53				17.62±8.38			
Th2	d0	11.20±1.31	0.027	0.027	0.946	23.19±6.94	0.599	0.524	0.720
	d7	27.04±7.73				19.44±3.48			
	d30	23.96±6.36				29.90±8.68			

Th17	d0	11.37±2.59	0.588	0.735	0.414	13.76±3.78	1.000	0.715	0.903
	d7	22.88±7.61				14.83±5.54			
	d30	12.52±2.93				13.84±4.06			
Th1/Th2	d0	0.64±0.13	0.623	0.635	0.216	0.93±0.28	0.489	0.389	0.389
	d7	0.74±0.18				0.77±0.18			
	d30	0.58±0.13				0.52±0.09			
Th17/Th2	d0	0.98±0.21	0.685	0.305	0.349	0.70±0.19	0.430	0.277	0.092
	d7	0.90±0.20				0.74±0.24			
	d30	0.60±0.09				0.54±0.13			
Th1+Th17/Th2	d0	1.62±0.30	0.906	0.444	0.244	1.63±0.43	0.679	0.188	0.110
	d7	1.64±0.32				1.51±0.37			
	d30	1.18±0.19				1.06±0.19			
Th1+Th17/ Th2+Th22	d0	1.05±0.24	0.722	0.839	0.340	1.14±0.27	0.847	0.208	0.095
	d7	1.00±0.15				1.07±0.18			
	d30	0.80±0.12				0.75±0.11			
Th1+Th17/ Th2+Th9+Th22	d0	1.01±0.22	0.787	0.910	0.349	1.05±0.26	0.709	0.268	0.107
	d7	0.97±0.15				1.01±0.18			
	d30	0.78±0.12				0.73±0.11			