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Effects of Vitamin D and Omega-3 Fatty Acids Co-Supplementation on Inflammatory Factors and Tumor Marker CEA in Colorectal Cancer Patients Undergoing Chemotherapy: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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ABSTRACT

Objectives: This study aimed to investigate the effects of vitamin D and omega-3 fatty acids co-supplementation on inflammatory factors and tumor marker CEA in colorectal cancer patients undergoing chemotherapy.

Methods: In this study, 81 patients with stage II or III colorectal cancer were randomly assigned into four groups: (1) control: receiving a vitamin D placebo, weekly + two omega-3 fatty acid placebo capsules, daily; (2) omega-3 fatty acid, receiving two omega-3 fatty acid capsules (each capsule containing 330 mg of omega-3 fatty acids), daily + a vitamin D placebo, weekly; (3) vitamin D, receiving a 50,000 IU vitamin D soft gel, weekly + two omega-3 fatty acid placebo capsules, daily; (4) co-supplementation, receiving a 50,000 IU vitamin D soft gel, weekly + two omega-3 fatty acids capsules, for 8 weeks. Before and after the intervention, serum levels of 25(OH)D, TNF-α, IL-1β, IL-6, IL-8, NF-kB activity, and tumor marker CEA, were measured.

Results: After 8 weeks of intervention, patients who received combined vitamin D and omega-3 fatty acids supplements compared with omega-3, vitamin D, and placebo had significantly decreased TNF-α, and IL-1β ($P < .05$). In addition, serum levels of TNF-α, IL-1β, IL-6, IL-8, and tumor marker CEA were decreased significantly in omega-3, vitamin D, and co-supplementation of them, compared with baseline. NF-kB activity was decreased significantly in vitamin D and co-supplementation groups, compared with baseline. Regarding CEA, there was no significant difference between the four groups at the end of intervention ($P > .05$).

Conclusion: Results show that co-supplementation of vitamin D and omega-3 fatty acids co-supplementation, in colorectal cancer patients have beneficial impacts on inflammation and tumor marker CEA.

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Introduction

Colorectal cancer (CRC) is a major public health concern. Epidemiological studies have shown that CRC is the third most common cancer worldwide (1), and is ranked as the fourth leading cause of deaths from cancer worldwide according to the World Health Organization (2). Inflammation promotes cancer development via creating an inflammatory microenvironment during tumor formation. The inflammatory and immunosuppressive cytokines released from these cells not only enhance proliferation, invasion, angiogenesis, and metastasis but also repress the host's immune system and accelerate tumor growth and development in CRC (3).

It has been indicated that absolute carcinoembryonic antigen (CEA) level is a strong independent prognostic factor for patients with CRC. Tumor marker levels, such as CEA, may demonstrate tumor biological activity (4).

During colon tumor progression, specific molecular processes have been targeted for chemopreventive intervention; such as chronic inflammation, proliferation and differentiation, apoptosis, cell surface growth factor receptors, angiogenesis, and metastasis

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(3). In spite of understanding the process and mechanism in colon carcinogenesis, present therapies including surgery, chemotherapy, radiotherapy, and molecular-targeted therapy are still limited for advanced tumors (3). Hence, a growing amount of scientific consideration has been focused on investigating the potential of dietary factors for both prevention and control of CRC.

Among dietary agents, there are growing epidemiological, clinical, and experimental studies which demonstrate a protective impact of omega-3 polyunsaturated fatty acids (PUFAs, found in fish oil), including ecosapantaenoic acid (EPA; C20, omega-3) and docosahexaenoic acid (DHA; C22, omega-3), against the induction and progression of colon cancer (5–7). However, the results have been discrepant and the literature does not have a consensus on the dose and time of the fish oil supplementation (8,9).

Several other reports have also demonstrated positive impacts of combining vitamin D or its analogs with a variety of chemotherapeutic factors in the treatment of various cancers (10–12). Vitamin D receptor is present in cells and tissues contributed in calcium regulation and in a large variety of other cells including malignant cells (12,13). Remarkably, people with low vitamin D levels had a higher risk of developing CRC (14).

Given the importance of the investigation the role of vitamin D and omega-3 fatty acids as anti-cancer agents, and due to the impact of co-supplementation of them on clinical outcomes of patients with colorectal cancer is not clear, the aim of this study was to evaluate the effects of vitamin D and omega-3 fatty acids co-supplementation on some inflammatory biomarkers and tumor marker CEA in CRC patients.

Methods and Materials

The present study was a randomized, double-blind, placebo-controlled clinical trial. Recruitment took place in oncology clinic of Tehran Gastroenterology & Hepatology Center (TGHC), Iran, between April 2018 and March 2019. The protocol of this study was approved by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, is in conformity with the Declaration of Helsinki (approval number: IR.AJUMS.REC.1396.1077) and was registered at the Iranian Registry of Clinical Trials (IRCT registration number: IRCT 20180306038979N1) which is available at: http://irct.ir/user/trial/20288/view. Written informed consent was obtained from all patients.

Participants were 81 patients with stage II or III colorectal cancer aged 26–73 years. The inclusion criteria consisted of: patients with stage II or III colorectal cancer but without distant organ metastasis, and were candidate to receive 2-weekly cycles of chemotherapy; aged over 18 years; body mass index (BMI) in range of 18.5–30 kg/m^2; without diabetes, hypertension, renal, hepatic, and parathyroid disorders; were not taking vitamin D and/or omega-3 supplements and other vitamin-mineral supplements or parenteral nutrition; not allergic to fish and fish products; without history of having other cancers; with normal biochemical tests consisting leukocyte and platelet counts more than 3500/mm^3 and 100,000/mm^3, respectively, alanine aminotransferase and aspartate aminotransferase levels below the 2.5 times of upper limit range, total bilirubin concentration below 1.8 mg/dl, and serum level of creatinine under 1.3 mg/dl. We excluded the patients who were refusing to continue the chemotherapy treatment or participation in the study; and the subjects who had less than 90% compliance with the intervention.

Sample Size

The number of participants calculated according to the changes in TNF-α levels between the control and co-supplementation groups, and based on the study by Mohammadzadeh et al. (15). It was calculated with the use of fixed factor levels model (determining sample size for analysis of variance) (16) and considering sigma of 1.25, 90% power, α of 0.05. Finally, 20 patients were recruited for each group. To allow for attrition, 24 patients were recruited for each group. Collectively, the sample of 96 patients with stage II or III colorectal cancer was recruited.

Intervention and Randomization

The 81 patients, who met the criteria, were randomly allocated into four groups: (1) control ($n = 20$): receiving a vitamin D placebo, weekly + 2 omega-3 fatty acid placebo capsules, daily; (2) omega-3 fatty acid ($n = 20$), receiving 2 omega-3 fatty acid capsules (each capsule containing 330 mg of omega-3 fatty acids), daily + a vitamin D placebo, weekly; (3) vitamin D ($n = 21$), receiving a 50,000 IU vitamin D soft gel, weekly + 2 omega-3 fatty acid placebo capsules, daily; (4) co-supplementation ($n = 20$), receiving a 50,000 IU vitamin D soft gel, weekly + 2 omega-3 fatty acids capsules (each capsule containing 330 mg of omega-3 fatty acids), daily; (4), for 8 weeks. Each omega-3 fatty acid capsule (MorDHA VISION, Minami Nutrition, Belgium) contained 54 mg of EPA, 250 mg of DHA, 26 mg of other omega-3 fatty acids. 50,000 IU vitamin D soft gels were supplied by Zahravi Pharmaceutical

Company (Zahravi, Tabriz, Iran). Vitamin D placebo and omega-3 fatty acid placebo were containing oral paraffin and corn oil, respectively. Placebo soft gels were provided by Zahravi Pharmaceutical Company (Zahravi, Tabriz, Iran), that had been approved by Food and Drug Administration. The appearance of the placebo soft gel was indistinguishable in color, shape, size, and packaging, smell, and taste from vitamin D and omega-3 fatty acids soft gels. Patients received verbal and written counseling on how to consume the soft gels. Compliance was evaluated by capsule count every 2 weeks. The supplementation began 1 week before starting the first session of chemotherapy.

The patients were allocated randomly using a random number table; for this, a person who was not involved in the study protocol created the randomization list assigning patients to the vitamin D, omega-3, vitamin D, and omega-3 co-supplementation, or the placebo group. Vitamin D, omega-3, and placebo soft gels were placed in to unlabeled identical containers. The study leader labeled these containers with patient numbers using the randomization list. All investigators and patients were blinded to the random assignments.

Outcome Measures

A questionnaire about patients' demographic positions, medications, diseases, cancer history, and probable supplement and/or medications use was recorded at the beginning of the intervention.

Dietary intakes were investigated with a 24-h food recall for 3 days (2 week day and 1 weekend day), throughout the intervention and nutrient intakes were determined by using nutritionist-4 software.

Blood samples were collected after 12 h overnight fasting, before and 1 week after the intervention and serum was obtained by centrifugation at 800–1000 RPM for 10 min. Then serum 25(OH)D, inflammatory factors, and tumor marker CEA was measured. Serum 25(OH)D was measured by enzyme- linked immune sorbent assay (ELISA) and Euro Immun kit (Euro Immun, Germany). Serum levels of TNF-α, IL-1β, IL-6, and IL-8 were assessed by ELISA and Bender Med kit (Bender Med, Germany). NF-kB activity was measured by ELISA and Crystal day kit (Crystal day, China). Serum concentrations of tumor marker CEA were measured by ELISA and CanAg kit (CanAg, Italy).

Statistical Analysis

All statistical analyses were performed using SPSS (Version 22.0; SPSS Inc., Chicago, IL). The normal distribution of variables was examined and confirmed by Kolmogorov-Smirnov test. All results were expressed as mean ± SD. Categorical variables are demonstrated as frequencies and percentages. Chi-square test was used to assess the differences between categorical variable. The baseline differences of mean values were tested using one-way analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to identify any differences between the four groups at the end of study, adjusting for baseline values and covariates. The comparison of mean values was done within groups after the intervention using paired sample t tests. Bonferroni was used to pairwise comparisons for the values after the intervention. $P < .05$ was considered statistically significant.

Results

In this study, 96 patients were recruited. In the vitamin D group, three patients were excluded from the study, because of changing the chemotherapy plan. In other groups, 12 patients (four patients in each of the groups) were excluded due to withdraw, noncompliance with the intervention, and changing the chemotherapy plan. Finally, statistical analysis was performed on 81 patients (Figure 1).

Forty-six patients (57%) were male and 35 patients (43%) were female. The mean age of participants was 58 ± 11 years old. General characteristics of patients are shown in Table 1. There were no significant differences between the four groups in age, sex, stage of disease, location of tumor, height, weight, and body mass index (BMI) at baseline ($P \geq .05$). Based on the 3-day dietary recalls obtained throughout the intervention, no statistically significant difference was seen between the four groups in terms of dietary intakes of calorie, macronutrients, and micronutrients including vitamin D (data not shown). No side effects were reported after supplementation of vitamin D and omega-3 fatty acids in colorectal cancer patients through the study.

Regarding serum 25(OH)-D, there was no difference between the four groups, at baseline ($P = .81$). However, a significant difference in serum levels of 25(OH)-D was seen between the four groups ($P < .001$) after the intervention (Table 2). Serum 25(OH)-D decreased significantly after the intervention in the control ($P < .001$) and omega-3 fatty acids ($P < .01$) groups, and increased significantly in the vitamin D ($P < .001$) and co-supplementation ($P < .001$) groups at this time (Table 2). Pairwise comparisons showed that there were significant differences in serum levels of 25(OH)-D between the control with vitamin D

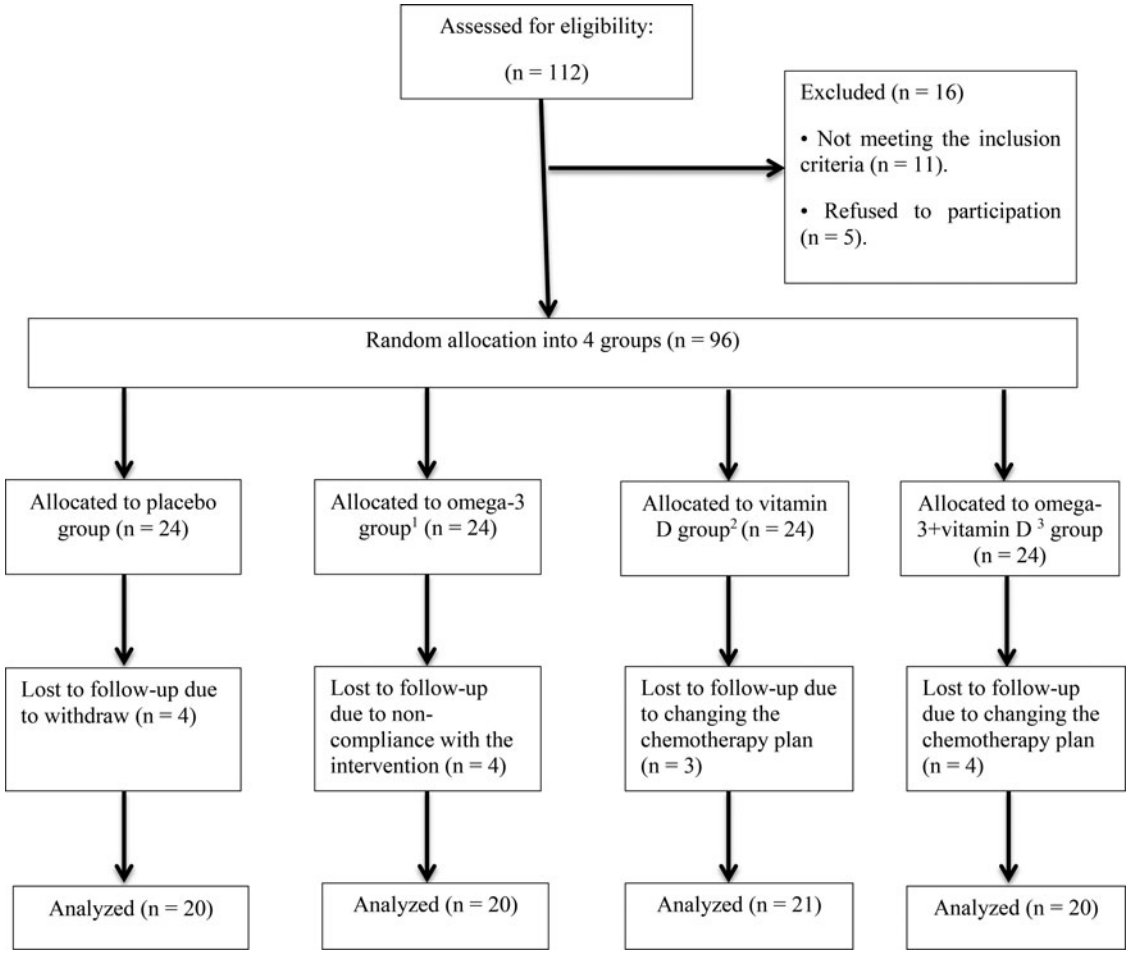

Figure 1. Flow diagram of the study. [1]Patients received 330 mg omega-3 fatty acid capsule twice a day plus a vitamin D placebo every week; [2]patients received a 50,000 IU vitamin every week plus two omega-3 placebo capsules; [3]patients received a 50,000 IU vitamin D soft gel every week plus 330 mg omega-3 fatty acids twice a day.

Table 1. Baseline characteristics of patients.

Variable	C ($n = 20$)	I1 ($n = 20$)	I2 ($n = 21$)	I3 ($n = 20$)	P
Age (years)[a]	59.90 ± 8.75	56.75 ± 10.60	56.90 ± 12.45	57.15 ± 10.17	.75*
Sex (M/F) (n)	8/12	12/8	16/5	10/10	.11**
Stage of disease (II/III) (n)	11/9	10/10	8/13	9/11	.75**
Tumor location (C/R) (n)	17/3	14/6	12/9	13/7	.27**
Fish frequency (n)	0	0	0	0	1.00**
Height (cm)	163.70 ± 8.48	167.95 ± 7.16	168.04 ± 8.70	164.05 ± 7.00	.14*
Weight (kg)	68.77 ± 11.99	68.40 ± 9.82	72.95 ± 11.11	67.91 ± 9.76	.48*
BMI (kg/m^2)	25.41 ± 3.55	24.26 ± 2.91	25.73 ± 3.53	25.13 ± 3.31	.54*

C: control group; I1: omega-3 group; I2: vitamin D group; I3: vitamin D-omega 3 co-supplementation.
[a]Data are presented as mean ± SD.
*Obtained from analysis of variance test (ANOVA).
**Obtained from chi-square test.
M/F: male/female; C/R: colon/rectum; BMI: body mass index.
Fish frequency: number of patients in the group who ate fish more than once a week.

($P < .001$) and co-supplementation ($P < .001$) groups (Table 3). In addition, the comparisons demonstrated differences in serum 25(OH)-D between the omega-3 with vitamin D ($P < .001$) and co-supplementation ($P < .001$) groups (Table 3).

No significant differences were seen between the four groups in terms of serum levels of TNF-α ($P = .73$), IL-1β ($P = .79$), IL-6 ($P = .88$), IL-8 ($P = .88$), and NF-kB activity ($P = .73$) at baseline (Table 2). Adjusting for baseline values, ANCOVA showed significant differences between the four groups in TNF-α ($P = .03$), IL-1β ($P = .03$), and IL-8 ($P < .001$) levels. However the mean changes in serum levels of IL-6, IL-8, and TNF-α showed significant differences between the four groups, after the intervention (in all differences, $P < .01$) (Table 2). The

Table 2. Serum levels of inflammatory factors of the patients at baseline and postintervention.[1]

Variable	Time	C ($n=20$)	I1 ($n=20$)	I2 ($n=21$)	I3 ($n=20$)	*P*
25(OH)-D (ng/ml)	Baseline	11.20 ± 9.89	12.74 ± 8.89	11.56 ± 9.84	9.72 ± 10.71	.81[a]
	Postintervention	6.21 ± 7.80	10.16 ± 9.96	37.30 ± 7.33	37.07 ± 9.42	<.01[b]
	Change	−4.99 ± 3.81	−2.58 ± 7.32	25.73 ± 6.08	27.35 ± 7.71	<.001[b]
P[c]		<.001	<.01	<.001	<.001	
NF-kB (OD)	Baseline	0.24 ± 0.02	0.25 ± 0.02	0.24 ± 0.03	0.24 ± 0.01	.73[a]
	Postintervention	0.24 ± 0.03	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.02[b]	.36[b]
	Change	−0.02 ± 0.01	−0.01 ± 0.01	−0.01 ± 0.01	−0.01 ± 0.02	<.001[b]
P[c]		.24	.44	<.01	<.001	
TNF-α (pg/ml)	Baseline	6.68 ± 2.82	5.93 ± 1.31	5.98 ± 2.62	6.13 ± 2.33	.73[a]
	Postintervention	6.76 ± 2.88	5.32 ± 1.41	4.93 ± 2.34	4.86 ± 2.12	.03[b]
	Change	0.08 ± 0.65	−0.61 ± 0.68	−1.04 ± 0.71	−1.27 ± 0.99	<.01[b]
P[c]		.94	<.001	<.001	<.001	
IL-1β (pg/ml)	Baseline	2.53 ± 0.81	2.42 ± 0.77	2.34 ± 0.65	2.31 ± 0.73	.79[a]
	Postintervention	2.49 ± 0.80	2.23 ± 0.76	2.26 ± 0.66	2.15 ± 0.69	.03[b]
	Change	−0.03 ± 0.18	−0.18 ± 0.22	−0.07 ± 0.11	−0.15 ± 0.21	.05[b]
P[c]		.42	<.01	<.01	<.01	
IL-6 (pg/ml)	Baseline	41.98 ± 51.40	32.01 ± 34.01	38.82 ± 32.98	39.04 ± 44.08	.88[a]
	Posrintervention	41.64 ± 51.26	29.98 ± 33.13	33.54 ± 28.80	34.56 ± 40.70	.81[b]
	Change	−0.34 ± 2.60	−2.03 ± 2.74	−5.27 ± 5.30	−4.48 ± 4.40	<.01[b]
P[c]		.39	<.01	<.001	<.001	
IL-8 (pg/ml)	Baseline	5.81 ± 0.13	5.82 ± 0.14	5.84 ± 0.14	5.81 ± 0.13	.88[a]
	Posrintervention	5.73 ± 0.17	5.72 ± 0.15	5.73 ± 0.20	5.74 ± 0.27	<.001[b]
	Change	−0.07 ± 0.12	−0.09 ± 0.11	−0.10 ± 0.13	−0.34 ± 0.31	<.01[b]
P[c]		.01	<.01	<.01	<.001	

C: control group; I1: omega-3 group; I2: vitamin D group; I3: vitamin D-omega 3 co-supplementation.
NF-kB: Nuclear factor kB; OD: optical density; TNF-α: Tumor necrosis factor α; CRP: C- reactive protein; IL-1β: Interluekin-1β; IL-6: Interluekin-6; IL-8: Interluekin-8.
[1]The results are expressed as mean ± SD.
[a]Difference between groups at baseline, *P* value is reported based on One-way ANOVA.
[b]Difference within groups at the end of the intervention, *P* value is reported based on analysis of covariance (ANCOVA).
[c]Within groups difference, *P* value is reported based on Paired *t* test.

Table 3. Pairwise comparisons for inflammatory factors and tumor marker CEA after the intervention.

Group	25(OH)-D (ng/ml)	NF-kB (OD)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	CEA (ng/ml)
C (I), I1 (J)							
Mean difference (I-J)	−2.91	0.01	0.76	0.26	2.19	0.01	0.19
P[a]	.62	1.00	.01	1.00	.26	1.00	1.00
C (I), I2 (J)							
Mean difference (I-J)	−30.84	0.02	1.19	0.22	5.09	−0.01	0.18
P[a]	<.001	1.00	<.001	1.00	<.001	1.00	1.00
C (I), I3 (J)							
Mean difference (I-J)	−31.86	0.01	1.40	0.34	4.28	0.26	0.33
P[a]	<.001	.62	<.001	.88	<.01	<.01	.34
I2 (I), I1 (J)							
Mean difference (I-J)	27.93	−0.01	−0.42	0.03	−2.89	0.01	0.01
P[a]	<.001	1.00	.42	.22	.04	1.00	1.00
I2 (I), I3 (J)							
Mean difference (I-J)	−1.02	0.01	0.20	0.11	−0.80	0.26	0.15
P[a]	1.00	1.00	1.00	.22	1.00	<.01	1.00
I1 (I), I3 (J)							
Mean difference (I-J)	−28.95	0.01	0.63	0.08	2.09	0.25	0.13
P[a]	<.001	.87	.05	1.00	.33	<.01	1.00

C: control group; I1: omega-3 group; I2: vitamin D group; I3: vitamin D-omega 3 co-supplementation.
NF-kB: Nuclear factor kB; OD: optical density; TNF-α: Tumor necrosis factor α; CRP: C reactive protein; IL-1β: Interluekin-1β; IL-6: Interluekin-6, IL-8: Interluekin-8.
CEA: carcinoembryonic antigen.
[a]Adjusted for multiple comparisons: Bonferroni.

comparisons within groups according to difference between the baseline and after the intervention values (Table 2) showed that all inflammatory biomarkers decreased in the three intervention groups, but NF-kB activity reduced in vitamin D and co-supplementation groups. Pairwise comparisons, after the intervention, showed that there were significant differences in levels of TNF-α and IL-6 between the vitamin D with the control groups (in both differences, $P<.001$), (Table 3). It was found that there were significant differences in TNF-α, IL-6, and IL-8 levels between the control with co-supplementation groups (for IL-6 and IL-8, $P<.01$, for TNF-α, $P<.001$) (Table 3). In addition, significant difference was seen in TNF-α ($P=.01$)

between the control and omega-3 groups (Table 3). Moreover, there was a significant difference in IL-8 between the co-supplementation with omega-3 and vitamin D groups (in both differences, $P < .01$).

No differences were seen between the four groups in terms of NF-kB activity and tumor marker CEA, at baseline and after the intervention (Tables 2 and 4). In addition, pairwise comparisons demonstrated that there were no significant differences in NF-kB and CEA between the groups, at the end of the study (Table 3). However, the comparisons within groups according to difference between the baseline and after the intervention (Table 4) demonstrated a reduction in serum levels of CEA in four groups.

Discussion

To the best of our knowledge, this is the first randomized double-blind, placebo-controlled clinical trial that investigate the effects of vitamin D or omega-3 fatty acids supplementation and co-supplementation of them, during an 8-week period parallel to chemotherapy, on inflammatory factors and tumor marker CEA in patients with colorectal cancer. We found that vitamin D (50,000 IU, weekly) and omega-3 fatty acids (660 mg, daily) co-supplementation for 8 weeks in stage II or III colorectal cancer patients undergoing chemotherapy had beneficial effects on inflammation and tumor marker CEA. The decrease in outdoor activities as a result of cancer or cancer treatment may be responsible for the reduction of 25(OH)D in control and omega-3 fatty acids groups.

A variety of cytokines (TNF-α, IL-1β, and IL-6) and other factors such as NFκB and cyclooxygenase-2 (COX-2) are involved in the incidence of inflammation resulting from cancer and cancer treatment (15). It has been indicated that inflammation has an important effect on progression, invasion, metastasis, chemoresistance, and radioresistance in colorectal cancer (15). Hence, today, many studies are conducted with the aim of the development of new anti-inflammatory therapeutic approaches for suppressing their synthesis or action (17), especially by dietary supplements (18) such as vitamin D and omega-3 fatty acids.

A study was carried out by Read et al. (19), in which patients with stage IV colorectal cancer were recruited to receive 2.18 g EPA and 0.92 g DHA for 9 weeks (3 weeks prior to chemotherapy and 6 weeks during chemotherapy). In these patients, CRP elevated significantly between baseline and week 3; while, it reduced significantly to baseline levels during the chemotherapy phase. Previous studies in healthy subjects have shown a relationship between n-3 polyunsaturated fatty acids and low serum levels of pro-inflammatory biomarkers (IL-6, TNF-α, IL-1, and CRP), supporting the belief that n-3 fatty acids may be useful in patients with diseases characterized by active inflammation (20). In another clinical trial (8), 23 patients with colorectal cancer undergoing chemotherapy randomly distributed in two groups. The supplemented group received 2 g fish oil containing 600 mg of EPA and DHA for 9 weeks. The patients in the supplemented group demonstrated a significant reduce in CRP; however, no significant results were found for interleukins, possibly due to the number of patients within the trial or because of the quantity of EPA + DHA.

In vitro studies have demonstrated that treatment of different colorectal cancer cell lines with DHA induce growth-inhibitory impacts on these cells (21,22). A study was conducted by Ahangar et al. (23), in which colorectal cancer cells were treated to different concentrations of DHA and proliferation, survivin expression, caspase-3 activation, and apoptosis were investigated by different cellular and molecular techniques. DHA was a repressor of survivin expression, elevate caspase-3 and apoptosis in colorectal cancer cells and may suggest a novel approach for the treatment of colorectal cancer at the early stage of tumor initiation. In this study, DHA also inhibited the growth of LS174T cells in a time-dependent and dose-dependent trend, which is in agreement with the results obtained by Dommels et al. (24), demonstrated a dose-dependent reduce in proliferation of Caco-2 cells by treatment with DHA.

Inflammation, an improper immunologic activity, is an ordinary characteristic of cancer. The presence and immensity of a chronic systemic inflammatory response may generate progressive nutritional decrease (8). Oral supplementation of different fatty acids changes the human immune cell and alters behavior and the immune response, such as its inflammatory component (25).

Polyunsaturated fatty acids have been demonstrated to have an inhibitory impact on pancreatic carcinoma cell lines in vitro (26). These impacts may occur via cell cycle arrest and the induction of apoptosis (27). EPA also reduces the speed of the growth of experimental tumors in mice (28). However, serial tumor imaging in colorectal cancer is difficult to interpret and expensive and was not performed in the current study.

Another evidence to indicate the anti- colorectal cancer impacts of omega-3 PUFAs (29–31), suggesting

Table 4. Serum levels of tumor marker CEA in the patients at baseline and post intervention.[a]

Variable	Time	C ($n=20$)	I1 ($n=20$)	I2 ($n=21$)	I3 ($n=20$)	P
CEA (ng/ml)	Baseline	2.89 ± 1.71	2.56 ± 1.67	2.84 ± 1.65	2.88 ± 1.36	.90[b]
	Posrintervention	2.66 ± 1.74	2.15 ± 1.63	2.43 ± 1.65	2.32 ± 1.42	.29[c]
	Change	−0.23 ± 0.32	−0.41 ± 0.68	−0.40 ± 0.54	−0.56 ± 0.59	.30[c]
P[d]		<.01	.01	<.01	<.001	

C: control group; I1: omega-3 group; I2: vitamin D group; I3: vitamin D-omega 3 co-supplementation.
CEA: carcinoembryonic antigen.
[a]The results are expressed as mean ± SD.
[b]Difference between groups at baseline, P value is reported based on One-way ANOVA.
[c]Difference within groups at the end of the intervention, P value is reported based on analysis of covariance (ANCOVA).
[d]Within groups difference, P value is reported based on Paired t test.

a theory to describe the health benefits of EPA and DHA. The suggested mechanism is that the omega-3 PUFAs can compete with arachidonic acid (ARA, 20:4 ω-6) at almost every stage of eicosanoid biogenesis, resulting to decrease the formation of ARA-derived eicosanoids that are predominantly pro-inflammatory and/or pro-tumorigenic, and elevated formation of EPA- and DHA-derived metabolites which have beneficial impacts (32). In addition, many studies support that omega-3 PUFAs decrease the risks of colon inflammation (33–35). However, there are discrepant results from animal and human studies, which demonstrated that omega-3 PUFAs had no impact (36) or detrimental impacts (37), making it inconvenient to implement omega-3 PUFAs for disease prevention. Based on some studies, polymorphisms in the genes encoding enzymes in cytochrome P450 (CYP) pathway may impact the metabolism of omega-3 PUFAs (38,39), affecting the generation of bioactive lipid metabolites, and thereby involving to observed inter-individual variations to omega-3 PUFA supplementation (39). Other probable explanations for the inconsistent findings include the variation in timing of exposure to omega-3 PUFAs in tumorigenesis, and the effects of additional factors that may be present in human cohorts but absent in experimental models (40).

On the other hand, several studies have demonstrated that $1,25(OH)_2D$ suppresses the expression of pro-inflammatory cytokines including interleukin (IL)-1, IL-2, IL-6, and IL-8 (41,42). Chronic inflammation is related to elevated production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 which contribute to carcinogenesis via impacts on cell proliferation, apoptosis, differentiation, and angiogenesis (43). In patients with colon cancer, these pro-inflammatory cytokines are positively related to elevated cancer growth, higher neoplastic grade, and elevated risk of mortality (43). Vitamin D supplementation has been related to reduced circulating pro-inflammatory cytokines in colorectal adenomas patients (44). Some studies utilizing mouse models of colitis have indicated that vitamin D can be useful in preventing or improving inflammation and clinical disease (45,46). In animal study with the aim of the investigation the potential synergistic chemopreventive impacts of vitamin D (100 IU/kg/day) and metformin (120 mg/kg/day) against the development of early colon malignancy, indicated that combined use of vitamin D and metformin was more beneficial, than each agent alone, in decreasing colorectal tumor formation (47). $1,25(OH)_2D$, the active form of vitamin D, signals via the vitamin D receptor (VDR), and then interferes with some other signaling pathways, which may partially mediate its anti-tumor impacts (47). In a cohort of stage III colon cancer patients recruited in a clinical trial of postoperative chemotherapy, higher levels of 25(OH)D was related to reduced recurrence and improved survival (48). These findings are in agreement with previous studies in CRC supporting an improved outcome with higher vitamin D levels (49). In a placebo-controlled, randomized trial of vitamin D (1100 IU) and calcium (1400 mg) supplementation in women, Lappe et al. (50) reported a statistically significant reduce in the cancer incidence with supplementation, with 21 cancers in the vitamin D and calcium co-supplementation group, 32 cancers in the calcium group, and 38 cancers in the placebo group. The other randomized trial of vitamin D and cancer was Women's Health Initiative (WHI), which used a lower dose of vitamin D (400 IU) in a sample of women with considerably lower baseline vitamin D levels (51). The study reported no significant impact of vitamin D intervention on colorectal cancer incidence but found a highly significant inverse association between baseline 25(OH)D and incident cancer risk (51,52). However, the intervention dose was only 400 IU/day, and the supplement adherence was only about 50%. The Women's Health Initiative CaD (WHI CaD) was a 7-y, randomized, placebo-controlled trial of CaD (1 g Ca/400 IU vitamin D, daily) in postmenopausal women. The incidence of total cancer (excluding nonmelanoma skin cancers), breast and colorectal cancers was evaluated. The results demonstrated that for women in the WHI CaD who were not taking personal calcium or vitamin D supplements at randomization, CaD decreased the

risk of total, breast, and colorectal cancers (53). Moreover, in a nationwide, randomized, placebo-controlled trial of vitamin D_3 at a dose of 2000 IU per day and marine omega-3 fatty acids at a dose of 1 g per day for the prevention of cancer and cardiovascular disease among men 50 years of age or older and women 55 years of age or older in the United States. Primary outcomes were invasive cancer of any type and major cardiovascular events. Secondary outcomes included site-specific cancers, death from cancer, and additional cardiovascular events. According to the results of the study, during a median follow-up of 5.3 years, cancer was diagnosed in 1617 participants (793 in the vitamin D group and 824 in the placebo group; hazard ratio, 0.96; 95% confidence interval [CI], 0.88–1.06; $P = .47$). In the analyses of secondary outcomes, the hazard ratios were as follows: for death from cancer (341 deaths), 0.83 (95% CI, 0.67–1.02); for breast cancer, 1.02 (95% CI, 0.79–1.31); for prostate cancer, 0.88 (95% CI, 0.72–1.07); for colorectal cancer, 1.09 (95% CI, 0.73–1.62). Hence, supplementation with vitamin D did not result in a lower incidence of invasive cancer than placebo (54). In a pooled analysis of two randomized clinical trials ($n = 1129$, $n = 2196$) and a prospective cohort (n = 1713) to examine a broad range of 25(OH)D concentrations. The outcome was diagnosis of breast cancer during the observation periods (median: 4.0 years). Multivariate Cox regression showed that women with 25(OH)D concentrations ≥60 ng/ml had an 80% lower risk of breast cancer than women with concentrations <20 ng/ml (HR = 0.20, $P = .03$) (55). Additionally, Data from two cohorts representing different median 25(OH)D concentrations were pooled to afford a broader range of 25(OH)D concentrations than either cohort alone: the Lappe cohort ($n = 1169$), a randomized clinical trial cohort (median 25(OH)D = 30 ng/ml) and the GrassrootsHealth cohort ($n = 1135$), a prospective cohort (median 25(OH)D = 48 ng/ml). Cancer incidence over a multi-year period (median: 3.9 years) was compared according to 25(OH)D concentration. Cancer incidence was lower at higher concentrations of 25(OH)D. Women with 25(OH)D concentrations ≥40 ng/ml had a 67% lower risk of cancer than women with concentrations <20 ng/ml (HR = 0.33, 95% CI = 0.12–0.90) (56).

In vitro and in vivo studies have indicated growth inhibition and differentiation of colon malignant cell lines and xenografts through administration of $1,25(OH)_2D$ (57,58), and rat models of colorectal cancer kept on a $1,25(OH)_2D$ diet developed fewer metastases in compared to control rats (59).

There are several other mechanisms by which vitamin D exposure may impact survival after the diagnosis of colorectal cancer. Connecting epidemiologic studies to a biologic mechanism for colorectal cancer pathogenesis, preclinical studies have demonstrated that VDR and 1α-hydroxylase are present in both normal and cancerous colon cells (60,61). Activation of VDR via $1,25(OH)_2D$ leads to differentiation and apoptosis, and inhibits proliferation, angiogenesis, and metastatic potential (48). Vitamin D may also impact CRC biology via alternative mechanisms, such as modifying systemic inflammation and cellular immunity (48). In vitro data indicates that vitamin D is able to repress MAPK activity and subsequent pro-inflammatory generation via the upregulation of MAPK phosphatase-1 (62) and NFκB signaling via the upregulation of IκBα, an inhibitor of NFκB activation (62), or by the reduced expression of the NFκB component RelB which can result to suppression of dendritic cell differentiation and maturation (62). Based on the data, it was proposed a model where vitamin D represses inflammation through reducing p38MAPK activation in lamina propria cells, leading to reduce pro-inflammatory cytokine production by those cells, which in turn reduces NFκB activation in colonic epithelial cells (62). In addition, $1,25(OH)_2D$ is synthesized by the enzyme 25-hydroxyvitamin D_3 1-α-hydroxylase (CYP27B1) (47), and is deteriorated by the enzyme 25-hydroxyvitamin D_3 24-hydroxylase (CYP24A1) (47). It was found that the expression of CYP27B1 and VDR in colorectal tumors were reduced, vitamin D_3 administration elevated the expression of CYP27B1 and VDR (47).

In a study with the aim of the investigation of the effect of combination of omega-3 PUFAs and vitamin D on breast carcinogenesis, Yang et al. (63) evaluated the growth of MCF-7 (ER^+, PR^+), SK-BR-3 ($HER2^+$), and MDA-MB-231 (triple negative) cell lines after 48 h exposure to different dose of omega-3 PUFAs and/or vitamin D_3. It was shown that EPA and DHA combined with vitamin D_3 considerably decreased cell survival compared to the single treatment in three cell lines. Combination of omega-3 PUFAs and vitamin D_3 elevated breast cancer cell apoptosis. In the study, decreased total poly-ADP-ribose polymerase (PARP) protein and down-regulated caspase activity were observed in three subtype breast cancer cell lines after the combined treatment, and cleaved form of PARP was observed only in combined treatment MCF-7 cells, demonstrating that various signaling pathways mediate the cell apoptosis in the three cell lines. Moreover, it was found that combined treatment of

omega-3 PUFAs and vitamin D_3 resulted to more cell death, proposing the impact of vitamin D_3 on breast cancer cells is amplified by omega-3 PUFAs via unknown pathways. The combination of omega-3 PUFAs and vitamin D_3 had stronger impact on cell apoptosis among three subtypes of breast cancer cell lines. Bcl-2 and total PARP protein levels were reduced in combined treatment MCF-7 and SK-BR-3 cells.

Overall, vitamin D plus omega-3 fatty acids supplementation, for 8 weeks in stage II or III colorectal cancer patients undergoing chemotherapy, have a strong synergistic impact on inflammation and tumor marker CEA. A specific nutritional intervention, including the aforementioned one, leading to decrease inflammatory biomarkers, to ameliorate immune responsiveness and consequently to ameliorate the quality of life, can be a successful strategy for colon cancer therapy. We suggest confirmation of these findings can be obtained by further, larger studies, with longer follow-up period.

The current study was the first clinical trial that investigated the effects of vitamin D and omega-3 polyunsaturated fatty acids co-supplementation on inflammatory biomarkers and tumor marker CEA in patients with stage II or III colorectal cancer during chemotherapy. However, the main limitation of our study is the lack of measurements of fatty acids fractions at the study baseline and at the end of trial because of budget limitations. In addition, we evaluated the early clinical outcomes of colorectal cancer. Further studies, with longer follow-up periods, are required. As our study has been carried out in patients with colorectal cancer, the results cannot be extended to patients with other types of cancers.

Conclusion

Vitamin D (50,000 IU/week) plus omega-3 fatty acids (660 mg/day) supplementation, for 8 weeks in stage II or III colorectal cancer patients undergoing chemotherapy, have beneficial impacts on inflammation and tumor marker CEA.

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No potential conflict of interest was reported by the authors.

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Authors' contributions

FH, BA, MI, and MV designed this study. BA and MI participated in the conduct of the study. KA-A analyzed the data. BA drafted the manuscript. FH, MI, and MV critically revised the manuscript. All authors read and approved the final manuscript.

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