Title:

Omega-3 polyunsaturated fatty acids supplementation improve clinical symptoms in patients with covid-19: A randomized clinical trial

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DISCLOSURE

The authors of this study declare that they each have no conflict of interest.

Abstract

Aims: We hypothesized that omega-3 fatty acids would be an appropriate adjunct therapy for alleviating the inflammatory response and clinical manifestation in hospitalized patients with covid-19 disease.

Methods: This was a single-blind randomized controlled trial in Amir-Alam hospital in Tehran. Thirty adult men and women diagnosed with covid-19 were allocated to either control group (receiving Hydroxychloroquine) or intervention group (receiving Hydroxychloroquine plus 2 grams of DHA+EPA) for 2 weeks. Primary outcome of the intervention including CRP, ESR as well as clinical symptoms including body pain, fatigue, appetite and olfactory and secondary outcomes including liver enzymes were determined at the baseline and after omega-3 supplementation. Clinical signs were measured using self-reported questionnaires. There were commercial kits for determination of CRP and liver enzymes concentrations in the serum of

patients. For determination of ESR automated hematology analyzer was applied. The study of "Comparison of the effectiveness of omega-3 and Hydroxychloroquine on Inflammatory factors, liver enzymes and clinical symptoms in diabetic COVID-19 patients" was registered in Iranian Registry of Clinical Trials (IRCT) with ID number: IRCT20200511047399N1

Results: In comparison to control group, patients receiving omega-3 indicated favorable changes in all clinical symptoms except for olfactory (($p<0.001$ for body pain and fatigue, $p= 0.03$ for appetite and $p=0.21$ for olfactory). Reducing effects of omega-3 supplementation compared to control group were also observed in the levels of ESR and CRP after treatment ($p<0.001$ for CRP and $p=0.02$ for ESR). However, no between group differences in the liver enzymes serum concentrations were observed after supplementation ($p>0.05$).

Conclusion: Current observations are very promising and indicate that supplementation with moderate dosages of omega-3 fatty acids may be beneficial in the management of inflammation-mediated clinical symptoms in covid-19 patients.

WHAT'S KNOWN?

Until now, several clinical trials have demonstrated Omega-3 as a treatment for inflammation in ARDS patients which is a common manifestation of covid-19. In addition, despite some contradicting findings, administering omega-3 PUFAs has shown promising results in alleviation of fatigue, body pain and disabilities in physical senses such as appetite, olfactory. There is currently no clinical trial that investigate the effect of Omega-3 supplementation on various symptoms of covid-19.

WHAT'S NEW?

In accordance to our study results, 2-week treatment with 2 g of W3 is likely to reduce inflammation. However, there were not significant changes in sense of olfactory. There is a certain need for further clinical trials in this issue to confirm our findings.

1. Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV) was initially documented in 2002 when it was isolated from the respiratory secretions of some patients with atypical pneumonia (1-3). The phrase Covid-19 refers to the previously unrecognized virus which seems to be related to prior SARS-CoV subtypes but it is unique with regard to its genetic sequence (4-6). Since the identification of Covid-19 in December 2019 in Wuhan, Hubei, China, the world has experienced the third global outbreak due to coronavirus contamination (7, 8).

According to world health organization (WHO) reports until August 2020 more than 700 thousand people have died due to infection with covid-19. After settling in the respiratory tracts, covid-19 infection begins pneumonia and inflammatory-related symptoms such as dyspnea, fatigue, weakness, anorexia, impaired taste and smell sensations and generalized body pain (9-13). High-risk populations include elderly, people with chronic inflammatory conditions such as cardiovascular diseases, diabetes and kidney failure (12, 14). The well-defined process of pneumonia usually starts from monocytes and macrophages migration to the lung airways and then transmits into inflammatory phenotype involved in adverse outcomes (13, 15). The inflammatory phenotype is characterized by the reduced blood concentrations of lymphocytes in particular natural killer (NK) cells and elevated monocytes, macrophages, CD4+ and Th17 cells which in the next step contribute to extremely high secretion of inflammatory mediators such as C-reactive protein (CRP) and elevated erythrocyte sedimentation rate (ESR), an established marker of acute systemic inflammation (16-18). Thus, therapeutic such as Hydroxychloroquine which focus on modulating inflammation can yield impressive benefits in covid-19-related manifestations (19, 20).

Omega-3 fatty acids (W3 FAs) have received much interest from nutrition world due to their long history of use as a therapy for inflammatory situations (21). The evidence to date, has declared that W3 fatty acids, may exert their anti-inflammatory effect by their ability to disfavor arachidonic acid oxygenation (22). As demonstrated by the in vitro and in vivo studies, such a mechanism may require dosages as high as 3 grams of W3 fatty acids (23). Currently, there is no clinical trial regarding the benefits of omega-3 therapy to reduce severity of covid-19 symptoms. However, to date, promising results have indicated the alleviating effect of omega-3 supplementation on excessive secretion of inflammatory cytokines which is a feature of covid-19 infection (24, 25). In addition, W3 supplementation improved fatigue, pain, impaired smell sense or anorexia in people with various disease background (26-29) that in some cases these improvements were supposed to be associated with reduced inflammation (27). A recent review also reported the probable protective effect of omega-3 treatment against Covid-19 symptoms progression based on experimental researches (30). Although, such improvements were not observed in in some trials (31, 32). Considering all these findings, the targets of this clinical trial were to examine both inflammatory and clinical symptoms in DM patients with covid-19 after W3 supplementation.

2. Method

2.1. Study design and participants

The current investigation was a controlled single-blinded trial, performed at Amir-Alam Hospital in Tehran.

30 adults (aged>18) with certain DM and with Body mass index (BMI) >18.5 enrolled in this study from February 2020 to April 2020 after had been diagnosed with Covid-19 according to the decision of the relevant physician. When they were recruited in this trial, the patients were treated Hydroxychloroquine. Pregnant, lactating and menopause women, those with diagnosis of International Normalized Ratio (INR) or platelet abnormalities and known history of liver, kidney or thyroid abnormalities, pancreatitis, any malignancy as well as smoking, drug and alcohol abuse and other conditions causing inflammatory state were not included in this study. We also excluded individuals who had regularly consumed W3 supplements during 3 months before study baseline.

Furthermore, during the intervention, screening visits was conducted to remove subjects who either lacked cooperation or reported any allergic reactions to study supplements or had any changes in their diet, prescribed therapies and supplements.

Approval to conduct the study was received from Ethics Committee of the Tehran University of Medical Sciences (TUMS) as ID: IR.TUMS.VCR.REC.1399.440 and it also, was registered with the Iranian Registry of Clinical Trials (ID: IRCT20200511047399N1) before commencing recruitment. Moreover, written consent for participation was received from all subjects after they had obtained complete information about the study.

2.2. Assessment of anthropometric, physical and clinical variables

After the demographic information including disease background, age, gender, race, education and physical activity were reported by each subject, participants were asked to respond questions regarding their self-interpretation about their senses of fatigue, body-pain, appetite, smell and taste. For assessment of mentioned clinical variables there were self-reported questionnaires, in which the number of 1 and 10 were indicative of the lowest and highest degree of each clinical variable, respectively.

The measurements of these clinical data were also repeated after 2-week Omega-3 supplementation. Total score for each subject's physical activity was also based on International Physical Activity Questionnaire.

For measurement of weight and height via Seca falcon scales (Seca, Germany), there was a situation that contained standard clothing and standing position for each participant. Moreover, the determination of the waist circumference (cm) was at the thinnest area of the waist and via a non-stretchable standard tape. The BMI was then calculated from the measured weight and height via the following formula: Weight/height^2 (kg/m2).

2.3. Assessment of laboratory variables

At the morning of the baseline day and the day after 2- week intervention period, a total of 10 ml of venous blood samples were collected from all recruited patients while they were in the fasting state.

Serum samples were then separated from all tubes following centrifugation at 3000-4000 RPM for 10 min and maintained at −20°C in the next step until laboratory assays.

The trial's primary laboratory outcomes included CRP, ESR and clinical manifestations which were measured twice before and after Omega-3 supplementation.

For serum CRP determination, immunoturbidimetric method (Pars Azmun, Iran) was applied.

ESR was also measured using automated erythrocyte sedimentation rate analyzer (ELECTA LAB S.r.l, Via Balzella, Italy). The Liver enzymes included Alanine transaminase (ALT), Aspartate transaminase (AST) or Alkaline phosphatase (ALP) were measured using a HITACHI 717 Automatic Analyzer, BoehringerMannheim Diagnostics company, Canada

2.4. Supplementation

Following clinical and anthropometric evaluation, 30 subjects were randomly (1:1) allocated to receive either W3 (3 capsules containing 670 mg EPA and DHA + 2 capsules containing 400 mg Hydroxychloroquine) or 2 capsules containing 400 mg Hydroxychloroquine for 2 weeks. Stratified Randomization method was based on BMI and gender to ensure equal assignment in both groups. Furthermore, during the trial period, compliance with intervention was considered as more than 90% consumption of W3, Hydroxychloroquine in both active and control groups.

2.5. Statistical analysis

All Statistical analyses in current RCT were conducted via Statistical Package for Social Sciences (SPSS) version 22 and on according to intention-to-treat (ITT) basis. To determine the distribution form of variables, Kolmogorov-Smirnov test was used. ΔCT values for the all study variables were summarized using their mean and the standard deviation (SD). Normally distributed quantitative and qualitative outcomes were compared between two groups based on one-way ANOVA and Chi-square tests, respectively.

The ANCOVA test was also applied to compare the study variables without the effect of confounding factors. If the variables were abnormal in distribution, we conducted Mann–Whitney U test for analysis. For each of these outcomes, we estimated the p-value<0.05 as significant.

3. Results

The initial and final number of patients in both W3 and control group are presented in figure 1. Of 39 eligible covid-19 patients, 5 individuals in actively treated group and 4 individuals in control group did not participate due to gastrointestinal problems, changing their prescribed drugs or lack of cooperation. Characteristics of entered patients before starting supplementation are also presented in Table 1. It was observed that all baseline characteristic was well balanced between two groups (p>0.05). The mean baseline BMI of all patients was about 26, representative of their overweight before supplementation. Analysis of baseline characteristics also declared that there was a high prevalence of male gender as well as low physical activity among patients (Table 1).
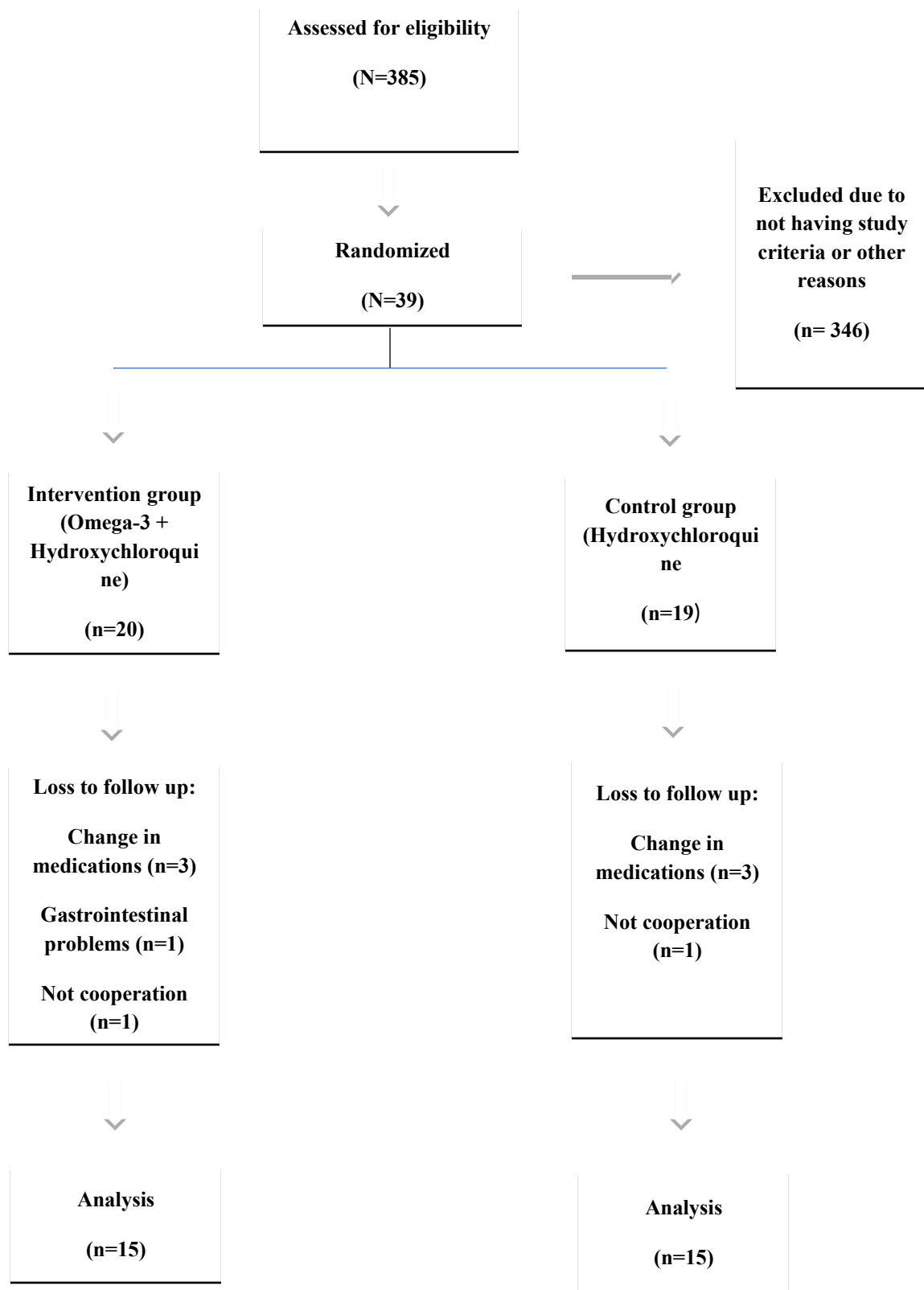
Assessed for eligibility

(N=385)

Randomized

(N=39)

Excluded due to not having study criteria or other reasons

(n= 346)

Intervention group (Omega-3 + Hydroxychloroquine)

(n=20)

Control group (Hydroxychloroquine

(n=19)

Loss to follow up:

Change in medications (n=3)

Gastrointestinal problems (n=1)

Not cooperation (n=1)

Loss to follow up:

Change in medications (n=3)

Not cooperation (n=1)

Analysis

(n=15)

Analysis

(n=15)

Figure 1. Flow chart for the trial

Table 1. Baseline demographic and clinical information participants in study groups

Characteristics		*Hydroxychloroquine* *(n=15)*	*Hydroxychloroquine* *+ Omega-3* *(n=15)*	P value [a]
Sex (male/ female)		9/6	9/6	
Age (year)		67.06± 2.28	66.46± 2.89	0.87
Weight (kg)		74.06± 2.22	75.53± 3.27	0.74
Height (m)		1.68± 0.01	1.66± 0.01	0.57
BMI (kg/m²)		26.13± 0.73	27.01± 0.94	0.47
Physical Activity (n/%)	Low	11 (73.33%)	12 (80%)	0.44 [b]
	Moderate	2 (13.33%)	2 (13.33%)	
	Sever	2(13.33%)	1 (6.66%)	
Systolic pressure (mmHg)		132.37 ± 0.81	134.69 ± 0.31	0.86
Diastolic pressure (mmHg)		85.63 ± 0.71	84.07 ± 0.15	0.87
Hb1AC (%)		6.60± 0.09	6.58± 0.11	0.93
Diabetes Duration (years)		5.5± 0.71	4.75± 0.76	0.43
Drug treatment (n)	Insulin	5	6	0.71
	Metformin	6	5	
	Sulfonylureas	2	1	
	Combined	2	3	
[a] Independent sample test				
[b] chi-square				

P value <0.05. Each value represents mean ± SE.

Above 90% of study supplements were consumed by all participants in both groups; hence compliance rate in this RCT was considered 100%. No side effects were reported following Omega-3 supplementation in patients with covid-19 throughout the intervention.

Comparing the post-treatment to the baseline in active group, using pair T-test, the level of ESR and concentrations of CRP in serum were found to be differentially decreased (p-value<0.05).

Furthermore, the finding of independent sample T test revealed that the reduction for both ESR and CRP was meaningfully different between the active and the control group, favoring active group (p<0.001 and p=0.02 for CRP and ESR respectively).

There were also significant decreased levels of fatigue and body pain and increased levels of appetite as result of the 2-week supplementation with W3 in comparison to control group (p<0.001 for fatigue and pain, p=0.03 for appetite).

When compared to the control group, however, the patients who consumed W3 did not demonstrate a significant reduction in olfactory dysfunction as well as serum levels of liver enzymes including AST, ALT and ALKP (p-value>0.05). Table 2 and 3 summarizes each outcome's values in the Omega-3 and control groups based on their mean and standard deviation (SD).

Table 2. Inflammatory parameters and liver enzymes of participants in study groups

Parameter		*Hydroxychloroquine* *n=15*	*Hydroxychloroquine* *+ Omega-3* *n=15*	P value [a]	P value [b]
CRP (mg/L)	Before	88.26± 2.72	92.80± 4.74	0.48	
	After	41.46± 8.57	25.06± 1.39	<0.001	<0.001
	Differences	-46±3.66	-67.73± 73	0.03	
ESR (mm/h)	Before	55.86± 4.17	59.26± 3.16	0.26	

Parameter		Hydroxychloroquine n=15	Hydroxychloroquine + Omega-3	P value [a]	P value [b]
	After	33.33± 2.49	26.33± 2.4	0.06	0.02
	Differences	-22.53± 2.75	-32.93±4.05	0.04	
ALT (U/L)	Before	36.46± 2.78	33.53± 2.34	0.53	
	After	24.28± 1.69	22.65± 1.63	0.71	0.67
	Differences	-12.18± 2.55	-10.88± 2.50	0.83	
AST (U/L)	Before	46.40± 5.08	44.40± 3.18	0.83	
	After	36.99± 2.57	33.10± 2.68	0.26	0.32
	Differences	-9.40± 3.29	-11.29± 4.07	0.46	
ALKP (U/L)	Before	213.15± 16.09	225.93± 13.53	0.36	
	After	186.93± 7.87	178.86± 7.18	0.43	0.64
	Differences	-34.15± 17.57	-47.06± 10.76	0.20	

[a] Mann-Whitney U test

[b] ANCOVA test

P value <0.05. Each value represents mean ± SE.

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALKP: Alkaline phosphatase; CRP, C-reactive protein, ESR, Erythrocyte sedimentation rate

Table 3. Clinical sign of participants in study groups

Parameter	Hydroxychloroquine n=15	Hydroxychloroquine + Omega-3	P value [a]	P value [b]

			n=15		
Pain (scoring from 1-10)	Before	8.76± 0.23	8.58± 0.25	0.60	
	After	6.43± 0.20	4.26± 0.21	<0.001	<0.001
	Differences	-2.33± 0.21	-4.32± 0.26	<0.001	
Fatigue and weakness (scoring from 1-10)	Before	9.16± 0.18	9.13± 0.19	0.9	
	After	6.36± 0.30	4.06±0.16	<0.001	<0.001
	Differences	-2.80± 1.37	-5.06± 0.21	<0.001	
Olfactory (scoring from 1-10)	Before	8.33± 0.74	8.20± 0.73	0.89	
	After	8.46± 0.74	8.86± 0.66	0.69	0.21
	Differences	0.13± 0.19	0.66± 0.37	0.21	
Appetite (scoring from 1-10)	Before	5.13± 0.35	5.66±0.46	0.36	
	After	6.76± 0.28	7.86± 0.33	0.01	0.03
	Differences	1.63± 0.53	2.20±0.35	0.38	

[a] Independent sample test

[b] ANCOVA test

P value <0.05. Each value represents mean ± SE.

4. Discussion

This is the first randomized controlled trial for examining the influence of W3 FAs on clinical symptoms and inflammatory parameters in DM patients with Covid-19. In our study, there was a high prevalence of BMI>25, low physical activity as well as male gender among participants which makes the hypothesize that male gender, obesity and poor physical activity might increase the possibility of infection with covid-19 (33).

Considering inflammatory markers first, in comparison to control group, W3 treatment led to significant reduction in serum levels of ESR and hs-CRP in participants. We have applied ESR and CRP as markers of systemic inflammation, because of the various mechanisms by which acute inflammatory diseases such as viral infections lead to rapid growths in these markers (34, 35).

During body reaction to inflammatory states, NF-κB activation and other pathways trigger secretion of inflammatory cytokines (36), among them IL-6, IL-1 and TNF-alpha reinforce CRP production (37, 38) and the generation of asymmetric and high molecular weight proteins such as fibrinogen which contributes to accelerated ESR (34).

W3 FAs can effectively suppress IL-6, IL-1 and TNF-alpha secretion in PBMCs by inhibiting NF-κB pathway (39), down-regulation of eicosanoid synthesis (40) and production of anti-inflammatory lipid metabolites including protectins and resolvins (41).

Although conflicting findings exists, most of studies investigating efficacy of W3 on ESR and CRP levels, have demonstrated similar results in different inflammatory condition such as patients with sepsis, kidney failure, systemic lupus erythematosus (SLE), migraine and cardiovascular diseases (CVD) (42-47). However our observations contrast with those from the Dichi et al's study, in which W3 supplementation led to accelerated Ulcerative Colitis (UC) disease characterized with elevated both CRP and ESR blood concentrations (48).

Furthermore, W3 supplementation could not reduce CRP level in the studies by Oliveira et al (49) and Muldoon et al (50) on healthy and HIV-infected adults. The reasons for this inconsistency might be the effects of low dose Omega-3 supplementation and different disease background.

We also discovered that compared to control group, short term W3 supplementation improved clinical manifestations of the disease except for olfactory dysfunction.

These changes were in agreement with the changes in inflammatory parameters. In fact, we hypothesized that alleviated CRP and ESR levels might explain a part of mechanisms by which W3 reduced covid-19 physical symptoms.

For example, overstimulation of anorexigenic melanocortin system by IL-6, IL-1 and TNF-alpha has been associated to the pathophysiology of appetite dysfunction in some types of cancers (51). As reported earlier, Omega-3 was found to alleviate IL-6, IL-1 and TNF-alpha production by both direct and indirect functions (52), providing the evidence that Omega-3 might improve appetite via stimulation of orexigenic pathways.

Omega-3 besides its anti-inflammatory effects has indicated widespread functions through which might exerts its protective roles against physical disorders in patients with covid-19.

Regarding body pain, DHA has an ability to promote glutaminergic and serotonergic synaptic activity, which inhibits histone deacetylation and pain progression (53, 54).

Moreover, it is believed that Omega-3 may exert its analgesic role via its reducing effect on D- and E-series resolvins as well as the derivatives of arachidonic acid. Such mechanisms are known to suppress activity of pain receptors such as TRPV1 (55, 56).

In relation to fatigue, another explanation also focuses on the amount of muscle function as a major issue in tiredness progression. It is biologically possible that W3 supplementation in covid-19 patients may have led to improvement in muscle function through enhancing membrane fluidity, acetylcholine sensitivity as well as attenuating protein catabolism (57-60).

The rationale for the current study was supported by numerous trials which have investigated whether W3 supplementation could improve fatigue, pain or anorexia in people with different disease background. In this context, Irving et al. performed an RCT of 1 year with 204 participants with Alzheimer's disease (AD) receiving doses of 1.7 g of DHA and o.6 g of EPA or placebo. Their data showed that both body weight and appetite improved in the intervention group compared to placebo group (28). Goncalves et al also reported the appetizing property of Omega-3 in samples of tumor-bearing rats as a consequence of activations in orexigenic pathways (28). Despite this known effect, few trials have introduced Omega-3 as an adjunct therapy for weight loss and suppressing appetite in people with obesity (31). This controversy could be attributed to the influence of the differences in severity and type of inflammation between people with obesity and people with other inflammatory disorders.

Concerning the body pain, the result of current study is in accordance with those reported in two trials in which short term supplementation with dosages of omega-3 near to ours, caused significant improvement in pain in patients suffering from nonsurgical face, neck and back pain (61, 62). Conversely, high-dose W3 supplementation for 24 weeks did not make the same result in Lustberg et al' study and there was not significant change in the severity of pain induced by aromatase inhibitor between W3 and placebo group (63). Taking these results together, it seems that the pain reduction caused by Omega-3 can be efficacious, when W3 is prescribed in lower dosage for a shorter time.

Furthermore, several studies have confirmed the robust protective effects of W3 with various dosages against fatigue progression in different inflammatory situations such as rheumatoid arthritis (RA), SLE and chronic fatigue syndrome (CFS) (64-66). Indeed, the results of one of these studies indicated that improvement in fatigue state was dependent to the reduction in CRP blood level after W3 supplementation, likely due to the positive association between fatigue and CRP (27).

The results from Torkildsen et al (32) and Peppone et al's (8) studies however did not demonstrate any change in sense of fatigue after W3 supplementation among patients with multiple sclerosis and Breast Cancer Survivors. The inconsistency between results of these studies shows the importance of the type of inflammatory disease background in the effect of various dosage of W3 supplementation on sense of fatigue.

W3 is also reported to improve or protect against olfactory dysfunction in experimental and human clinical trials (29, 67). Moreover, in models of Omega-3 depleted rats, there have been positive associations between W3 deficiency in the brain and olfactory dysfunction (68, 69). In the current study however, W3 did not make significant improvements in sense of smell compared to medications in the control group, which could be due to low number of covid-19 patients in our study.

Until now, the probable benefits of omega-3 supplementation to alleviate COVID-19 severity were according to documented in vivo and in vitro experimental studies (30). Our study indicated that 2-week treatment with 2 g of W3 was well tolerated and was likely to be clinically beneficial in diabetic patients with covid-19 who were suffering from fatigue, body pain and loss of appetite. In contrast, W3 supplementation did not influence on sense of olfactory as well as circulating levels of liver enzymes. Due to the small number of our participants and their underlying disease (DM), caution needs to be taken in generalizing the current results to all population with covid-19 disease. Furthermore, our results of favorable clinical improvements in W3 group could have changed if we had measured clinical variables by objective methods. Additional studies with larger sample size are required to confirm W3 as a confidant adjunct therapy for patients with covid-19.

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