Original article

Inflammatory biomarkers, antioxidant enzyme activities, and oxidative stress in Iranian male patients with type 2 diabetes mellitus: Effects of eicosapentaenoic acid and vitamin C supplementation

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BACKGROUND: Eicosapentaenoic acid (EPA) is the principal ω-3 fatty acid in marine oils. Significant differences have been reported in the serum levels of inflammatory biomarkers, malondialdehyde (MDA), and antioxidant enzymes activity between diabetic patients and controls after supplementation with ω3 fatty acids and antioxidants. Therefore, the aim of this study was to determine the effects of EPA and/or vitamin C, as an antioxidant, on serum levels of interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), MDA, and glutathione reductase activity in type 2 diabetic Iranian males. **METHODS:** This study was performed on 65 men with type 2 diabetes who aged 33-63 years. Venous blood samples were obtained from all participants at 8:00-9:00 a.m. after 10 hours of fasting, at the baseline and after the intervention. Subjects received 500 mg EPA and/or 200 mg vitamin C or placebo depending on their groups. For eight weeks, 15 participants received EPA supplements with vitamin C (Group 1), 16 took EPA supplements and vitamin C placebo (Group 2), 17 took EPA supplement placebo and vitamin C (Group 3), and 17 received EPA supplement placebo and vitamin C placebo (Group 4), daily. **RESULTS:** There were significant falls in TNF-α levels in Groups 1 and 2 ($p < 0.05$) and in MDA in Group 3 ($p < 0.05$). On the other hand, a significant increase was observed in MDA in Group 2 ($p < 0.01$) and in glutathione reductase activity in Groups 1 and 3 ($p < 0.05$). Moreover, according to Tukey's test, there were significant differences in IL-6 ($p < 0.01$) and glutathione reductase activity ($p < 0.05$) between Group 4 and other groups. Meanwhile, Tukey's test showed that TNF-α was significantly different between Group 1 and other groups ($p < 0.01$), Groups 2 and 3 ($p < 0.05$), and Groups 2 and 4 ($p < 0.05$). Finally, there were significant differences in MDA levels between Groups 1 and 2 ($p < 0.01$), Groups 1 and 4 ($p < 0.05$), Groups 2 and 3 ($p < 0.05$), Groups 2 and 4 ($p < 0.05$), and Groups 3 and 4 ($p < 0.05$) after 8 weeks of intervention. **CONCLUSIONS:** In summary, it is concluded that eight weeks of EPA + vitamin C supplementation improved inflammation and antioxidant status in male type-2 diabetic patients.

KEYWORDS: Eicosapentaenoic Acid, Vitamin C, Interleukin-6, Tumor Necrosis Factor Alpha, Malondialdehyde, Glutathione Reductase, Diabetes.

BACKGROUND

Type 2 diabetes mellitus (T2DM) is the most prevalent and serious metabolic disorder all over the world.[1] There are significant differences in the serum levels of inflammatory biomarkers between diabetic patients and controls. Elevated serum levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) among diabetics have been shown in several studies.[2-4] TNF-α has been reported to be associated with insulin resistance[5,6] and T2DM.[7,8]

On the other hand, T2DM is associated with increased oxidative stress, which may contribute to its complications.[9,10] An increase in oxidative stress occurs due to an increase in the production of free radicals or a reduction in antioxidant capacity. The adverse effects of free radicals are neutralized in vivo by a wide range of antioxidants such as vitamins C and E, glutathione, carotenoids, and antioxidant enzymes such as glutathione reductase.[11,12]

Some studies have revealed increased production of plasma lipid peroxidation products, like malondialdehyde (MDA), in diabetics compared to controls.[13,14] On the other hand, it has been reported that antioxidant capacity decreases in diabetic patients.[12]

The objective of this study was to find out if eicosapentaenoic acid (EPA) with or without ascorbic acid (as an antioxidant) will have any effects on serum inflammatory biomarkers, glutathione reductase activity, and serum MDA levels patients with T2DM.

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METHODS

This randomized double-blind placebo-controlled clinical trial was conducted on 65 men with T2DM who aged 33-63 years. Ethical approval was obtained from the Medical Ethics Committee of Tehran University of Medical Sciences (Tehran, Iran) and informed consents were obtained from all subjects. The participants were instructed not to take any omega-3 fatty acids and antioxidant supplements during and 2 weeks preceding the study. Patients were excluded in case of nephropathy, myocardial infarction, coronary bypass grafting, and inflammatory diseases.

Venous blood samples were obtained from all participants at 8:00-9:00 a.m., after 10 hours of fasting, at baseline and after the intervention. Subjects received 500 mg EPA and/or 200 mg vitamin C or placebo depending on their groups. For a period of 8 weeks, 15 participants received daily EPA supplements with vitamin C (group 1), 16 took EPA supplements and vitamin C placebo (group 2), 17 took EPA supplement placebo and vitamin C (group 3), and 17 received EPA supplement placebo and vitamin C placebo (group 4). EPA and EPA placebo softgels were supplied by Minami Nutrition (Belgium). Vitamin C and placebo softgels were obtained from Darou Pakhsh Pharma Chem. Co. (Iran). IL-6 and TNF-α enzyme-linked immunosorbent assay (ELISA) kits were from Bender Medsystems GmbH (Vienna, Austria).

Glutathione reductase was determined by the Sauberlich method.[15] Serum MDA levels were assessed using the method described by Satoh.[16]

Weight and height were measured with Seca standard tools and body mass index (BMI) was calculated according to the formula [weight (kg)/height^2 (m^2)].

Microsoft Excel was used for data handling. SPSS$_{10}$ (SPSS Inc., Chicago, IL, USA) and Stata$_7$ (Stata Corp., College Station, TX, USA) were used for statistical analyses. Values are expressed as mean ± standard deviation (SD) for each group. The significant level was set at $p < 0.05$.

RESULTS

A total number of 69 male diabetic patients with a mean age of 48 years (33-63 years) and mean BMI of 29.47 kg/m^2 (21-40 kg/m^2) were recruited. Finally, 65 participants completed the 8-week intervention. Mean plasma levels of IL-6, TNF-α, and MDA and glutathione reductase activity are presented in table 1. There were significant decreases in TNF-α in groups 1 and 2 ($p < 0.05$), and in MDA in group 3 ($p < 0.05$).

Table 1. Comparisons of parameters in the groups 1 [eicosapentaenoic acid (EPA) and vitamin C], 2 (EPA and vitamin C placebo), 3 (vitamin C and EPA placebo), and 4 (vitamin C placebo and EPA placebo) before and after 8 weeks of supplementation

Parameters	Group 1 (n = 15)			Group 2 (n = 16)			Group 3 (n = 17)			Group 4 (n = 17)		
	Baseline values	change	P-value (paired t-test)	Baseline values	change	P-value (paired t-test)	Baseline values	change	P-value (paired t-test)	Baseline values	change	P-value (paired t-test)
IL-6 (pg/ml)	16.81 ± 0.60	1.51 ± 0.63	0.07	16.90 ± 0.54	2.46 ± 0.43	0.06	15.55 ± 0.51	1.51 ± 0.57	0.10	17.37 ± 0.60	0.57 ± 0.70	0.70
TNF-α (pg/ml)	7.77 ± 0.38	2.32 ± 1.04	0.02	11.26 ± 1.10	2.26 ± 1.41	0.04	11.19 ± 0.99	1.07 ± 4.42	0.20	12.80 ± 1.04	0.40 ± 0.04	0.70
MDA (nmol/l)	1.30 ± 0.09	0.12 ± 0.46	0.10	1.65 ± 0.19	1.41 ± 1.27	0.01	2.05 ± 0.13	0.90 ± 0.16	0.03	1.46 ± 0.13	0.24 ± 0.13	0.10
Glutathione reductase (u/l)	1.98 ± 0.05	0.52 ± 0.08	0.01	2.15 ± 0.12	26.00 ± 0.05	0.10	2.00 ± 0.13	0.21 ± 0.09	0.01	1.95 ± 0.09	0.006 ± 0.005	0.70

IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; MDA: Malondialdehyde

Table 2. Multiple comparisons of parameters among groups 1 [eicosapentaenoic acid (EPA) and vitamin C], 2 (EPA and vitamin C placebo), 3 (vitamin C and EPA placebo), and 4 (vitamin C placebo and EPA placebo) after 8 weeks of supplementation

Parameters	Group 1 (n = 15)	Group 2 (n = 16)	Group 3 (n = 17)	Group 4 (n = 17)
IL-6 (pg/ml)*	14.03 ± 0.30	14.43 ± 0.34	15.30 ± 0.35	18.94 ± 0.17
TNF-α (pg/ml)**	7.77 ± 0.38	10.05 ± 0.54	12.80 ± 1.04	13.53 ± 0.74
MDA (nmol/l)***	1.14 ± 0.09	1.23 ± 0.04	1.12 ± 0.09	1.71 ± 0.04
Glutathione reductase (u/l)*	2.37 ± 0.13	2.42 ± 0.12	2.50 ± 0.07	1.95 ± 0.09

Results are expressed as the mean ± SD. IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; MDA: Malondialdehyde; * Significant differences between group 4 and other groups ($p < 0.01$) (Tukey's test); ** Significant differences between group 1 and other groups ($p < 0.01$), groups 2 and 3 ($p < 0.05$), groups 2 and 4 ($p < 0.05$) (Tukey's test); *** Significant differences between groups 1 and 2 ($p < 0.01$), groups 1 and 4 ($p < 0.05$), groups 2 and 3 ($p < 0.05$), groups 2 and 4 ($p < 0.05$), and groups 3 and 4 ($p < 0.05$) (Tukey's test)

In addition, significant increases in MDA were observed in group 2 ($p < 0.01$) and in glutathione reductase activity in groups 1 and 3 ($p < 0.05$). Moreover, according to Tukey's test, there were significant differences in IL-6 and glutathione reductase activity between group 4 and other groups ($p < 0.01$ and $p < 0.05$, respectively). Similarly, TNF-α levels were significantly different between group 1 and other groups ($p < 0.01$), groups 2 and 3 ($p < 0.05$), and groups 2 and 4 ($p < 0.05$). Finally, there were significant differences in MDA levels between groups 1 and 2 ($p < 0.01$), groups 1 and 4 ($p < 0.05$), groups 2 and 3 ($p < 0.05$), groups 2 and 4 ($p < 0.05$) and groups 3 and 4 ($p < 0.05$) after 8 weeks of intervention (Table 2).DISCUSSION

DISCUSSION

T2DM is accompanied by increased inflammatory cytokines.[2] Meanwhile, many studies have shown that T2DM may result in oxidative stress[9,10] that could be reversed with vitamin C. This randomized double-blind placebo-controlled clinical trial study on male patients with T2DM demonstrated a fall in TNF-α levels ($p < 0.01$) in groups 1 (EPA and vitamin C) and 2 (EPA and vitamin C placebo) before and after the intervention. Meanwhile, TNF-α was significantly different between group 1 and other groups ($p < 0.01$), groups 2 and 3 ($p < 0.05$), and groups 2 and 4 ($p < 0.05$) following 8 weeks of EPA supplementation. These findings suggest that EPA along with vitamin C can decrease the production of inflammatory cytokines. While some studies reported similar results,[17-19] others foundomega-3 fatty acids to be inefficient in reducing the production of inflammatory cytokines.[20]

EPA is an omega-3 fatty acid derived from marine oils that competitively inhibits the metabolism of arachidonic acid, an omega-6 polyunsaturated fatty acid (PUFA), and thus reduces the generation of inflammatory mediators[21] and the production of cytokines from inflammatory cells.[22] Consuming fish oil results in partial replacement of arachidonic acid in inflammatory cell membranes with EPA.[22,23] This response alone is a potentially beneficial anti-inflammatory effect of omega-3 PUFAs.

In this study, Tukey's test showed significant differences in glutathione reductase activity ($p < 0.05$) between group 4 (EPA placebo and vitamin C placebo) and other groups. Moreover, according to paired t-test, enzyme levels were higher in groups 1 and 3 ($p < 0.05$) after the intervention. Some investigators have shown that dietary supplementation with omega-3 PUFA results in increased glutathione reductase activity.[24,25] However, others demonstrated no effect in glutathione reductase activity after fish oil supplementation.[26]

The results of the present study also showed increased oxidative stress during EPA supplementation. In fact, plasma MDA levels in group 2 increased after the intervention ($p < 0.01$). Plasma MDA levels, however, were lower after supplementation in group 3 ($p < 0.05$). The results of several studies suggest that increased consumption of omega-3 fatty acids may result in an increased potential for oxidative stress in vivo.[27,28] This effect could be caused by more susceptibility of PUFAs to oxidation due to the higher numbers of double bounds.

In conclusion, this study showed that 8 weeks of EPA plus vitamin C supplementation in patients with T2DM enhanced the erythrocyte glutathione reductase activity. The intervention, however, reduced TNF-α levels. In addition, 8 weeks of EPA supplementation alone enhanced the serum levels of MDA and reduced the serum levels of TNF-α. Finally, 8 weeks of vitamin C supplementation alone reduced the serum levels of MDA and enhanced the glutathione reductase activity of erythrocytes.

The differences among reports about the effects of EPA and vitamin C supplementation on inflammatory markers and antioxidant status are probably methodological. The small number of studies and the different methods used for the assessment of inflammation and antioxidant status necessitate further studies.

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