Effect of omega-3 and ascorbic acid on inflammation markers in depressed shift workers in Shahid Tondgoyan Oil Refinery, Iran: a randomized double-blind placebo-controlled study

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The present study aimed to assess the effect of supplementation of omega-3 and/or vitamin C on serum interleukin-6 and high sensitivity C-reactive protein concentration and depression scores among shift workers in Shahid Tondgoyan oil refinery. The study design was randomized, double-blind, placebo-controlled, parallel trial. Totally 136 shift workers with a depression score ≥10 in 21-item Beck Depression Rating Scale were randomly assigned to receive omega-3 (180 mg eicosapentaenoic acid and 120 mg docosahexaenoic acid) or/and vitamin C 250 mg or placebo twice daily (with the same taste and shape as omega-3 and vitamin C) for 60 days in four groups. Depression score, interleukin-6 and high sensitivity C-reactive protein were measured at baseline and after 60 days. This study showed that supplementation of omega-3 plus vitamin C is associated with a decrease in depression score (p<0.05). Supplementation of omega-3 without vitamin C, is associated with a reduction in depression score (p<0.0001) and high sensitivity C-reactive protein concentration (p<0.01). Therefore omega-3 supplementation showed a better effect on reducing depression score and high sensitivity C-reactive protein, but supplementation of vitamin C along with omega-3 did not have significant effect on change in C-reactive protein level compared to omega-3 alone. (Registration number: IRCT20120218956N1)

Key Words: omega-3 fatty acids, vitamin C, interleukin-6 (IL-6), high sensitivity C-reactive protein (hs-CRP), depression

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hift work is defined as work primarily outside of normal daytime working hours (7 am to 6 pm).1 Nowadays shift work is an unavoidable part of the work system of many industries such as refineries. The oil refinery industry is an around-the-clock operation.2 More than 20 to 30 percent of workers are shift workers.3 Shift-workers are forced to work and sleep against normal chronic biological rhythms, they sleep at times that their organism is set to be active, and work when their physical and psychical effectiveness is generally low. These contradictions result in various disorders; most frequently, sleep disturbances.4

The rotational shift workers have difficulties adapting to their sleep/activity cycle, which is necessary for their work shift.5 Subjects working in rotating daytime schedule reported to have shorter sleep duration compared with when they are assigned to the morning shift. The prevalence of difficulty initiating sleep is higher in rotational shift workers as compared with regular day workers. Sleep deprivation and occupational stress leads to drowsiness and reduces neurobehavioral function and consequently the risk of depression may increase.6 Some studies found an association between both the parameters of poor sleep and symptoms of deep depression in male shift workers.7

Several authors reported an augmentation of cytokines after acute sleep deprivation and thereby an induction of pro-inflammatory immune responses.8,9 Cytokine levels correlate with fatigue and daytime somnolence. Therefore Vgontzas et al.10 called the Interleukin-6 (IL-6) cytokine “fatigue inducing cytokines”. Activation of inflammatory immune responses is marked by an increase in inflammatory markers, e.g. IL-6 or high sensitivity C-reactive protein (hs-CRP), which increases in sleep deprivation and are also considered as risk factors for most diseases, like depression.11

Studies on shift work and its effects on individuals’ health may lead to preventive interventions to reduce the risks.12 Modifications in diet can be one of the preventive interventions, although having an appropriate food habits is important in shift work, most of shift workers do not have a correct diet.13 Insufficient nutrient intake can influence brain function and cause clinical psychiatric symptoms.14 A diet consisting of antioxidants such as vitamins may be useful for shift workers and administration of antioxidant supplements, may be useful in reducing oxidative stress.

Omega-3 essential fatty acids (EFA) are of particular interest, as they are selectively concentrated in synaptic neuronal membranes and regulate immune functions that affect the central nervous system. There are some evidence showing that the amount of omega-3 or vitamin C in depressed or stressed individuals is low or omega-3 or vitamin C supplementation can improve depression and oxidative.15–17

The present study aimed to assess the effect of supplementation of omega-3 and vitamin C and their combination on serum IL-6 and hs-CRP concentration and depression scores among rotational shift workers. The study hypothesis is that there is a reduction in depression scores, the serum IL-6 and hs-CRP concentration in shift workers who received supplementation compared to those who did not.

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Materials and Methods

Participants. Men rotational shift workers from Tehran Shahid Tondgooyan Oil Refinery, aged 21–52 years, were enrolled into the study. There were a total of 456 shift workers (operational and firefighting refinery personnel) out of them 397 shift workers responded to the questionnaires. It should be noted that all of shift workers in Tehran Shahid Tondgooyan Oil Refinery were men and their program was 8 h back ward shifts (from night to morning), 4 nights (N), 3 off (O), 4 afternoons (A), 1 off (O), 4 mornings (M), respectively (NNNMOOAAAAOMMMM). Of these number those who met the inclusion criteria (n = 136) participated in the study.

The inclusion criteria was depression score ≥10 in 21-item Beck Depression Rating Scale, giving consent to participate in the study and wash-out periods of 2 month for antidepressant drugs and 2 weeks for supplementation prior to entering the study.

The exclusion criteria: Participants were excluded from the study if any of the following conditions were met: history of thyroid diseases, liver diseases, kidney diseases, diabetes, cancer and cardiovascular diseases. (After obtaining a detailed medical history, physical examination was performed by the interviewing physician), doing professional sports, smoking, consuming alcohol and substance abuse.

Trial design. This was a randomized, double-blind, placebo-controlled, and parallel-group clinical trial, conducted in Tehran, Iran. The assessment was done in health center of the refinery.

Interventions. The shift workers were randomly assigned to receive omega-3 (180 mg eicosapentaenoic acid and 120 mg docosahexaenoic acid) twice daily or/and vitamin C 250 mg twice daily or placebo twice daily (with the same taste and shape as omega-3 and vitamin C) for 60 days. Omega-3 and omega-3 placebo (paraffin oil) were supplied by Zahravi Co. (Tehran, Iran), vitamin C and their placebo were obtained from Osveh Co. (Tehran, Iran).

Outcomes. Data collection was performed using a self-administered general questionnaire. The general questionnaire included questions on age, marital status, work experience, shift work experience, education, sports, smoking, alcoholic drinks, narcotics and drugs abuse, the data about food intake in last 24 h was obtained by interview. 21-items Beck Depression Inventory, which was translated in Persian, was used to assess the depressive symptoms at baseline and also after 60 days. Diastolic and Systolic blood pressures were measured using a mercury sphygmomanometer with 5 mmHg precision from the right arm and after 10 min of resting in the sitting position. History of thyroid diseases, liver diseases, kidney diseases, diabetes, cardiovascular diseases (stroke, myocardial infarction, angina, atrial fibrillation, cardiac arrhythmia, cardiomyopathy, heart valve disease, aortic aneurysm and hypertension) and cancer were extracted from the participants’ occupational profiles. Weight and height were measured with Seca standard tools (Germany) with 0.1 cm and 100 g precision while the participants had least clothing and were barefoot and body mass index (BMI) was calculated from the formula [weight (kg)/height (m)²].

Eight ml of venous blood at sitting position and after fasting for 10–12 h was drawn by needle holder 21 in a gel-containing tube without anti-coagulant (Behdarou Company, Tehran, Iran) and centrifuged for 10 min at a speed of 1,500 rpm from each participant for measurement of IL-6 and hs-CRP at baseline and after 2 months. The extracted serum was transferred to micro tubes labeled for the participants and was kept at −70°C until the analysis time. IL-6 (pg/ml) and hs-CRP (mg/l) were measured in Tehran Nour Research Center using R&D (R&D Systems, Inc. Minneapolis, MN 55413, USA) kit in enzyme-linked immunosorbent method and quantitative diagnostic kit in immunoturbidimetry method produced by Pars Azmoon Company (Tehran, Iran). All samples were tested in the same run. The primary outcome was the difference in Beck Depression Index, IL-6 and CRP concentration from baseline to 2 months in the 4 study groups. The other primary outcome was the difference in BDI, IL-6 and CRP concentration from baseline to 2 months between groups (omega-3 and/or vitamin C and the placebo).

Randomization, allocation concealment and blinding. 136 shift workers using balanced block randomization method were divided into 4 groups (34 participants in each group at the initial phase). Omega-3 soft gel appears to be different with vitamin C pill so double-dummy technique was used in the study. Group 1 were on 2 omega-3 soft gels and 2 vitamin C pills daily regimen, group 2 were on 2 omega-3 soft gels and 2 vitamin C placebo pills daily regimen, group 3 were on 2 vitamin C pills and 2 omega-3 placebo soft gels and group 4 were on omega-3 placebo soft gels and 2 vitamin C placebo pills daily regimen. The regimen duration was 60 days. Random allocation was done by a third party and the participants, the researchers and laboratory personals were blind to the allocation.

Statistical analysis. All analyses were performed using statistical package for social sciences (SPSS) ver. 19.0 for windows (IBM Corporation, Armonk, NY) and data about food intake were analyzed by software FPII (ver. 2, food processor II Esha Research, Salem, OR). The categorical variables were reported as a frequency (percentage) and continuous variables were reported as mean (± SD). To compare the IL-6, hs-CRP and depression score before and after intervention among 4 groups, ANOVA and Tukey tests were used. Paired t test was used for comparison of IL-6, CRP and depression score pre- and post-treatment levels in each group. The significant level was set at p<0.05.

Ethical consideration. Review Board of Tarbiat Modares University approved the study. Ethical approval was obtained from the Medical Ethics Committee of Tarbiat Modares University in Tehran-Iran. The trial was conducted in line with the latest revision of the Declaration of Helsinki. All participants gave written consent.

Results

Fig. 1 shows the flow chart of the study participants inclusion and exclusion. 456 potentially eligible candidates were screened, 320 of them did not meet the inclusion criteria or met the exclusion criteria. 136 participants, with a mean age of 30.75 years (21–52 years), and mean BMI of 24.92 (15.9–34.3 kg/m²) were enrolled into the study and were randomly assigned to either omega-3 plus vitamin C (n = 34) or omega-3 plus vitamin C placebo (n = 34), omega-3 placebo plus vitamin C (n = 34), omega-3 placebo plus vitamin C placebo (n = 34), 126 of the participants completed the study (there was 10 cases of loss of follow up) (Fig. 1).

In group 1 (omega-3 and Vitamin C) one worker was excluded from the study due to lack of compliance, in group 2 (omega-3 and placebo vitamin C) three were lost due to disease and hospitalization, in group 3 (vitamin C and placebo omega-3) one was lost due to a broken leg and two did not consent to give the end blood sampling and one left the refinery, and in group 4 (placebo omega-3 and placebo vitamin C) one was lost because of change in his work schedule from shift work to non-shift work system and one did not consent to give the end blood sampling.

The result of ANOVA test showed there were no significant difference in age, work experience, shift work experience, systolic and diastolic pressure, BMI, depression score, serum IL-6 and CRP levels and food intake, at the baseline level between 4 groups (p>0.05), The Chi-Square test showed that there was no significant difference in history of family depression, history of depression, occupation, marital status, and educational level (p>0.05). Table 1 shows the baseline level of all the variables among different groups (Table 1). There were a significant corre-
lation between depression score and work experience ($r = 0.211$, $p < 0.05$) between depression score and shift work experience ($r = 0.218$, $p < 0.01$). There were a significant correlation between BMI and age ($r = 0.270$, $p < 0.01$).

**Clinical outcomes.** Table 2 shows mean ($\pm$ SD) of depression score, plasma levels of IL-6 and hs-CRP and depression score at the baseline level and the changes from baseline level after 60 days (end of study) among 4 groups. There was a significant reduction in the depression scores at the end of the 60 days [groups 1, 3 and 4 ($p<0.05$), and in group 2 ($p<0.0001$) respectively]. Post-intervention depression score (following 60 days of supplementation) was significantly different between groups 2 and 1 ($p<0.05$), and groups 2 and 4 ($p<0.05$) by using Tukey test. After intervention depression score decreased in groups 1, 2, 3 and 4, by 21.49%, 45.24%, 22.87% and 16.60%, respectively.

There were no significant difference in the serum IL-6 concentrations in groups 1, 2 and 3 ($p>0.05$) but there was a significant increase in group 4 ($p<0.05$), at the end of 60 days. Regarding serum hs-CRP concentration, the paired t test showed a significant reduction in the serum hs-CRP concentrations in groups 2 ($p<0.01$) and there was no significant difference in other groups ($p>0.05$).

Depression scores, at the end of the study were 11.18 ± 5.31 in group 1, 7.61 ± 5.16 in the group 2, 10.90 ± 5.90 in the group 3 and 11.75 ± 5.55 in the group 4. Serum IL-6 concentrations at the end of the study were 1.16 ± 0.92 pg/ml in group 1, 1.37 ± 1.44 pg/ml in group 2, 1.23 ± 0.99 pg/ml in group 3 and 1.78 ± 1.81 pg/ml in group 4. The serum hs-CRP concentrations at the end of the study were 0.85 ± 1.85 mg/l in group 1, 0.57 ± 0.77 mg/l in group 2, 0.73 ± 1.44 mg/l in group 3 and 1.63 ± 2.75 mg/l in group 4. Also ANOVA test showed that there were significant differences in depression scores change between groups after intervention, Tukey test showed that this difference was significant between group 2 and 1 ($p<0.05$), and groups 2 and 4 ($p<0.05$), there were no significant differences in IL-6 and hs-CRP change between groups after intervention ($p>0.05$).

**Discussion**

In Iran, few studies investigated the shift workers health status and those few were mostly conducted in hospitals, but studies in industries are rare. These studies have shown that Iranian shift workers are at risk for depression. The present study is the first Iranian clinical trial (based on the available literature) among shift workers that investigated the effect of ascorbic acid and omega-3 supplementation on reducing the stress and depression symptoms and on serum level of IL-6 and hs-CRP among 136 depressed rotational shift workers, which was conducted in Shahid Tondgoyan Oil Refinery (Tehran, Iran).

The results of this study showed a significant reduction in depression score in all groups after intervention. Reduction in depressive symptoms in the placebo group could be due to the nonspecific effect of treatment or other placebo effects. Improve-

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**Table 1.** Participants characteristics in different group

| Variable (mean ± SD)                             | Group 1 Omega-3 + Vitamin C (n = 34) | Group 2 Omega-3 + Placebo Vitamin C (n = 34) | Group 3 Placebo Omega-3 + Vitamin C (n = 34) | Group 4 Placebo Omega-3 + Placebo Vitamin C (n = 34) | p value |
|--------------------------------------------------|--------------------------------------|---------------------------------------------|---------------------------------------------|--------------------------------------------------|--------|
| Age (year)                                       | 29.47 ± 7.05                         | 31.71 ± 7.43                                | 30.71 ± 7.25                                | 31.12 ± 7.35                                      | 0.63   |
| Work duration (year)                             | 5.59 ± 6.65                          | 7.94 ± 8.03                                | 6.47 ± 5.32                                | 7.66 ± 6.76                                      | 0.45   |
| Shift Work duration (year)                       | 4.8 ± 5.44                           | 7.63 ± 8.83                                | 4.85 ± 4.09                                | 6.73 ± 7.16                                      | 0.13   |
| BMI (kg/m²)                                      | 23.85 ± 4.16                         | 25.42 ± 3.27                               | 25.56 ± 3.91                               | 24.85 ± 3.04                                     | 0.20   |
| IL-6 (pg/ml)                                     | 1.31 ± 1.52                          | 1.46 ± 1.17                                | 1.25 ± 1.45                                | 0.93 ± 0.54                                      | 0.34   |
| hs-CRP (mg/l)                                    | 1.17 ± 2.03                          | 1.27 ± 1.37                                | 0.84 ± 1.31                                | 1.96 ± 0.8                                       | 0.50   |
| Depression score (Beck Depression Inventory)     | 14.32 ± 3.68                         | 13.73 ± 4.20                               | 14.03 ± 4.19                               | 14.18 ± 3.35                                     | 0.93   |
ment in depression without treatment (active substances) can occur because of a mental feeling in patients about the treatment, some studies showed that this mental feeling can change Electroencephalography (EEG) in pre-frontal cortex (22) and the expected improvement can result reduction in depression symptoms. (23) In previous studies, also response to treatment has been reported in the placebo group in clinical trials related to depression. (24)

The findings of this study showed that the amount of reduction in depression score in group 2 was nearly twice than other groups, and Marangel et al. (25) showed that supplementation of a 4 g/day EPA plus DHA for 6 weeks reduced IL-6, significantly, however there are discrepancies among different studies in this regard, for example Mori et al. (26) showed that a 4 g/day EPA plus DHA for 6 weeks did not reduce IL-6, significantly. Therefore dose and duration of intervention may be an important factor for predicting the effect of omega-3 supplementation in decreasing the concentration level of IL-6.

Results of present study showed that omega-3 had a positive effect in reducing hs-CRP. This result is in accordance with the previous studies that showed omega-3 fats may help maintain healthy levels of hs-CRP. (30) Although some studies reported no association between the serum omega-3 fatty acid levels and serum CRP levels. (31) This inconsistency may be due to the methods of CRP assessment.

In conclusion, this study showed that 60 days of supplementation of omega-3 plus vitamin C is associated with a decrease in depression score (p<0.05). Supplementation of omega-3 without vitamin C, is associated with a reduction in depression score (p<0.0001) and hs-CRP blood concentration (p<0.01), vitamin C supplementation is associated with a decrease in depression score (p<0.05). Therefore omega-3 supplementation showed a better effect on reducing depression score and hs-CRP, but supplementation of vitamin C along with omega-3 did not have any significant effect on change in CRP level compared to omega-3 alone. Further studies with longer duration are required to determine the effects of supplementation of omega-3 with and without vitamin C on CRP concentration.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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Table 2. Mean (SD) of depression score, plasma levels of IL-6 and hs-CRP at the baseline, and comparison of depression score, IL-6 and hs-CRP changes from the baseline level in the four interventional groups

| Variables | Baseline (month 0) | Change (Baseline-end study) | p value for t test (baseline vs end point) | p value for ANOVA test for comparing changes (baseline vs end point) between group |
|-----------|-------------------|--------------------------|-------------------------------------|-------------------------------------------------------------------|
| IL-6 (pg/ml) |                    |                          |                                     |                                                                   |
| Omega-3 + Vitamin C (Group 1) | 1.321 ± 1.54 | 0.158 ± 1.52 | 0.555 | 0.26 |
| Omega-3 + Placebo Vitamin C (Group 2) | 1.487 ± 1.22 | 0.119 ± 1.81 | 0.717 | |
| Placebo Omega-3 + Vitamin C (Group 3) | 1.233 ± 1.53 | 0.003 ± 1.78 | 0.992 | |
| Placebo Omega-3 + Placebo Vitamin C (Group 4) | 0.928 ± 0.56 | 0.853± 1.80 | 0.012 | |
| hs-CRP (mg/l) |                    |                          |                                     |                                                                   |
| Omega-3 + Vitamin C (Group 1) | 1.203 ± 2.05 | 0.348 ± 1.35 | 0.149 | 0.113 |
| Omega-3 + Placebo Vitamin C (Group 2) | 1.229 ± 1.41 | 0.658 ± 1.04 | 0.001 | |
| Placebo Omega-3 + Vitamin C (Group 3) | 0.926 ± 1.37 | 0.200 ± 1.55 | 0.487 | |
| Placebo Omega-3 + Placebo Vitamin C (Group 4) | 0.844 ± 1.28 | 0.790± 2.56 | 0.091 | |
| Depression score |                    |                          |                                     | 0.015 |
| Omega-3 + Vitamin C (Group 1) | 14.242 ± 3.71 | 3.061 ± 6.83 | 0.015 | |
| Omega-3 + Placebo Vitamin C (Group 2) | 13.903 ± 4.63 | 6.290 ± 6.82 | 0.021 | |
| Placebo Omega-3 + Vitamin C (Group 3) | 14.133 ± 4.37 | 2.323 ± 7.08 | 0.018 | |
| Placebo Omega-3 + Placebo Vitamin C (Group 4) | 14.09 ± 3.42 | 2.34 ± 5.88 | 0.031 | |

*The amount of decrease.
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