

## Effect of Conjugated Linoleic Acid, Vitamin E, Alone or Combined on Immunity and Inflammatory Parameters in Adults with Active Rheumatoid Arthritis: A Randomized Controlled Trial

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### ABSTRACT

**Background:** Little information about the effects of conjugated linoleic acids (CLAs) on inflammation and immune function in humans is available. This study investigated the effects of CLAs, with and without Vitamin E on immunity and inflammatory parameters in adults with active rheumatoid arthritis (RA).

**Methods:** In a double-blind clinical trial, 78 patients were randomly divided into four groups, each group receiving one of the following daily supplement for 3 months; group C: 2.5 g CLAs, group E: 400 mg Vitamin E, group CE: CLAs plus Vitamin E, group P: Placebo. Cytokines, matrix metalloproteinase 3 (MMP-3) and citrullinated antibody (CCP-A) were measured by ELISA method and Vitamin E by high-performance liquid chromatography.

**Results:** Consider statistical methods there were no significant differences between groups in cytokines interleukin-2 (IL-2), IL-4, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$ , IL-2/IL-4, CCP-A white blood cells and neutrophils, lymphocyte, monocytes, and eosinophils numbers. TNF- $\alpha$  decreased in all groups, but its reduction was significant in group CE. IL-1 $\beta$  increased in groups P ( $P = 0.004$ ) and E ( $P = 0.041$ ) but the difference between group P and CE was significant. IL-4 decreased in groups C, CE and E ( $P = 0.03$ ,  $P = 0.03$ ,  $P = 0.07$  respectively). IL2 did not change significantly within groups. CCP-A increased in groups P ( $P = 0.035$ ) and E ( $P = 0.05$ ), while it decreased in groups CE ( $P = 0.034$ ). CCP-A and MMP-3 decrease were significant between groups P and CE. MMP-3 reduction was significant in group CE.

**Conclusions:** Co-supplementation CLAs and Vitamin E may be effective in the level of inflammatory markers in RA patients.

**Keywords:** Conjugated linoleic acids, immunity, inflammatory markers, rheumatoid arthritis, Vitamin E

### INTRODUCTION

Rheumatoid arthritis (RA) is a chronic relapsing inflammatory multisystem disease with synovial proliferation and destruction

of articular cartilage. It is the most common inflammatory arthritis, affecting approximately 0.5-1% of the general population worldwide.<sup>[1,2]</sup> RA has served as a useful model for the study of many inflammatory and immune-mediated diseases.<sup>[1]</sup> The exact cause of RA is not yet known. Recently, several studies have shown the possible role of reactive oxygen species (ROS) in the pathogenesis of RA.<sup>[3-9]</sup> The destructive reactions by ROS can be improved by antioxidants.<sup>[8]</sup> Antioxidants have beneficial effect for inhibition of inflammation related to neutrophil functions,<sup>[9]</sup> Vitamin E as a potent antioxidant has an ability to modulate host immune functions,<sup>[10]</sup> so it may have positive effect on patients who suffer from RA.<sup>[10-13]</sup>

Conjugated linoleic acids (CLAs) are naturally occurring isomers of linoleic acid found in meat and milk of grazing animals.<sup>[14]</sup> Their anti-inflammatory effects that have been shown can protect bones from damage.<sup>[14,15]</sup> The biological activities of CLAs, much attention has been due to their anticancer,<sup>[16]</sup> antiatherogenic<sup>[17,18]</sup> and antidiabetic effects,<sup>[19]</sup> as well as their effect on increasing the bone mass.<sup>[20]</sup> The role of CLAs in oxidative stress as an antioxidant has been investigated to explain its beneficial physiological effects.<sup>[21-28]</sup> In previous articles we reported the effects of CLAs on RA by a randomized, double-blind placebo-controlled trial. Pain and morning stiffness were significantly lower in the group taking CLA or CLA plus Vitamin E, compared to placebo the group after 12 weeks of supplementation. We have concluded that CLAs may improve clinical outcomes,<sup>[28]</sup> lower lipid peroxidation<sup>[29]</sup> without negative effects on lipid profile and fasting blood sugar in patients with RA.<sup>[30]</sup>

Other studies suggest that the CLA can also inhibit the cyclooxygenase (COX-1) and COX-2 pathway enzymes that enhance inflammation in diseases such as RA.<sup>[31-33]</sup> It is well established that lipids can influence both the inflammatory responses and T-cell functions.<sup>[34]</sup> CLAs have been reported to have anti-inflammatory and immune-regulatory effects on healthy animals.<sup>[35-37]</sup> In contrast to the animal studies, clinical trials in healthy persons have reported few effects of CLA on immune function.<sup>[34,38-44]</sup> There are controversial results about changing Plasma C-reactive protein values.<sup>[45-48]</sup> To our knowledge there has not been any study on CLAs and/or its combination with

Vitamin E on immunity and inflammation in patients with RA. The objective of the study was to determine the effects of oral administration of c9t11 and t10c12 CLA, Vitamin E supplement alone and in combination with Vitamin E supplement, on immunological and inflammatory parameters in active RA.

## METHODS

### Subjects

This study was a randomized, double-blind placebo-controlled trial that conducted in a 3 months period in patients with active RA. The inclusion criteria were ages between 18 and 69 years having RA for at least 2 years and fulfilling 1987 American College of Rheumatology criteria for RA.<sup>[49]</sup>

The exclusion criteria were: Taking vitamin and fatty acids/or mineral supplements, thyroid hormones, estrogens, progesterone, diuretics or  $\beta$ -blockers, abnormal renal and hepatic function, history of myocardial infarction, diabetes, hyperlipidemia and for the females, not being pregnant.

The subjects were fully informed of the purpose, procedures, and hazards of the trial and had the right to leave the trial at any time. Written consent was obtained from all patients. The study has been approved by the Ethics Committee on Human Experimentation of the Tehran University of Medical Sciences.

### Design

The patients were randomly allocated using Random Permuted Blocks procedure to one of following four treatment groups. All patients in either treatment groups received two capsules and one pearl daily for a period of 3 months as follow. For group C: Two CLA capsules (2 capsules 1.25 g/day that contained 80% CLA, equal 2 g 50:50 mix of cis - 9, trans - 11 and trans - 10, cis - 12 glycerinated CLA and one Vitamin E placebo pearl; group CE: Two CLA capsules and One Vitamin E pearl; group E: One Vitamin E ( $\alpha$  tocopherol) pearl (400 mg/day) and two CLA placebo capsules; for group P: Two CLA placebo capsules and one placebo pearl. Placebo capsules contained corn oil (CO) in replacing of Vitamin E and high oleic sunflower oil in replace of CLA.

Vitamin E and its placebo were specially prepared for this study by Zahravey Company, and CLA and its placebo by Lipid Nutrition Company.

### Biochemical analysis

**Blood sample collection:** A sample of 15 ml blood was obtained from each patient before the trial and at the end of it. The patients were fast for 12–14 h. Ethylene diamine tetra acetic acid as anticoagulant was used for plasma isolation.

Plasma  $\alpha$ -tocopherol was measured by high-performance liquid chromatography (Cuesta–Sanz method) with a C15 column and ultraviolet–visible detector.<sup>[50]</sup>

**Measurement of plasma inflammatory and immunity reactants:** Plasma cytokines were done by ELISA method<sup>[51–54]</sup> and by human high sensitivity ELISA kits from eBioscience Company [USA] (sensitivities for interleukin-2 (IL-2), IL-4, IL-1, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were 0.4, 0.1, 0.05, 0.13 pg/ml, respectively). Matrix metalloproteinase 3 (MMP-3) was measured by human ELISA Platinum kits from Ebioscience Company; sensitivity was 0.008 ng/ml. Citrullinated antibody (CPP-A) was measured by ELISA method for IgG Antibody citrullinated protein and kits produced by Genesis (England) company, and clinical sensitivity was 80%.<sup>[55]</sup>

Hematological values were determined by an automated blood counter (Beckman Coulter, Miami, USA).

Nutrients intakes were estimated using 24 h dietary recall questionnaire before and at the end of the trial for 3 days analyzed by Nutrition IV (San Bruno, CA, USA, Firsty Data Bank) software. The subjects were asked not to change their usual diets and physical activities throughout the study, and any changes in their medications were avoided if possible.

Compliance with the supplement intake was assessed by counting number of the capsules used and determining changes in the plasma  $\alpha$ -tocopherol.

### Statistics methods

Differences between the four groups were determined by ANOVA (one-way analysis of variance) for continuous data and the Chi-square test for group data. Log transformation was used to normalize the abnormal distributions. Differences

between before and after data in each group were determined with paired-sample *t*-test. If the distribution of a variable was not normal, Mann–Whitney U-test was used to compare the differences between two groups and Wilcoxon signed-rank test was performed for each group to compare mean values before and after intervention. ANOCVA were used to adjust the effect of confounding factors. Correlation was determined by Pearson test.  $P < 0.05$  was considered as statistically significant. (Version 18; SPSS Inc., Chicago, IL, USA) was used for data analysis. Quantitative values are reported as mean  $\pm$  standard deviation.

## RESULTS

Totally 102 subjects entered to the study, and 87 of them completed the study. Fifteen patients were excluded from the study due to either incomplete consumption of prescribed drugs (6 patients: 1 in each groups C [CLA] and CE [CLA + Vitamin E], 2 in each groups E [Vitamin E] and P [Placebo]), changing the dose of their antiinflammatory drugs (8 patients: 2 in each groups), or side-effects (1 patient in group C).

Table 1 shows demographic, anthropometric data for the 4 study groups at the baseline. There were no significant differences among the four groups at the beginning of the study regarding age, sex, BMI, daily intake of vitamin E, disease duration and diseases activity score ( $P > 0.05$ ). Also, the differences between drugs intake (NSAIDS, glucocorticoid and other disease-modifying antirheumatic drugs) weren't significant between groups ( $P > 0.05$ ).<sup>[28]</sup> The Plasma level of  $\alpha$ -tocopherol increased significantly in groups E and CE in contrast to the placebo group ( $P \leq 0.017$ ,  $P < 0.023$  respectively) [Table 2].

There were no significant differences between groups at the baseline in cytokines IL-2, IL-4, TNF- $\alpha$ , IL-1 $\beta$ , IL-2/IL-4 and citrullinated antibody variables [Tables 3] as well as white blood cell (WBC) [Tables 2 and 4]. In this study, significant changes were not seen in neutrophils, lymphocyte, monocytes, eosinophils numbers and BMI after treatment between groups. Decrease in WBC count was significant in group CLA plus Vitamin E, and the lymphocytes increased in group P ( $P \leq 0.05$ ) [Table 3].

Although TNF- $\alpha$  was reduced during the study in all groups, this reduction was significant

**Table 1:** Demographic, anthropometric, and clinical data for the four study groups at the baseline (mean±SD)

Variables	Group P (n=22)	Group C (n=22)	Group E (n=21)	Group CE (n=22)	P
Sex (female/male)	19/3	19/3	17/4	17/5	NS
Age (years)*	47.95±11.14	46.23±13.07	49.33±11.89	43.77±12.75	NS
Duration of RA (years)*	8.88±8.65	9.95±8.41	7.24±5.82	7.64±6.19	NS
BMI (kg/m <sup>2</sup> )	28.48.92±3.94	27.18±4.63	27.14±4.70	25.65±3.97	NS
Vitamin E intake (mg/day)*	7.9±2.44	8.75±2.31	7.78±3.50	8.80±3.44	NS
DAS28	4.35±0.95	4.63±1.26	4.59±1.11	4.52±1.08	NS

There were no significant differences between groups by ANOVA (for means) or Chi-square (for sex ratio). Group P=Placebo; Group C=CLAs; Group E=Vitamin E, Group CE=CLAs+Vitamin E. ANOVA=Analysis of variance, CLAs=Conjugated linoleic acids, BMI=Body mass index, SD=Standard deviation, RA=Rheumatoid arthritis, NS= Non significant

**Table 2:** The plasma levels of CCP-A, MMP-3, CRP and Vitamin E in patients with active RA before and after 3 months supplementation (mean±SD)

Variables	Group P (n=22)	Group C (n=22)	Group E (n=21)	Group CE (n=22)	P*
CCP-A (pg/ml)					
Before	28.62±14.13	50.93±62.05	30.23±39.43	49.41±50.88	0.002
After	48.44±46.36	45.06±49.08	46.16±48.32	31.38±26.1	
	P=0.035*	NS	P=0.055	P=0.034	
MMP-3 (ng/ml)					
Before	65.48±38.9	72.93±40.41	84±44.9 <sup>b</sup>	91.95±57.97 <sup>a</sup>	0.018
After	87.40±53.53	69.55±48.29	72.85±54.97	67.28±42.74	
	P=0.059	NS	NS	P=0.019	
Vitamin E (µg/ml)					
Before	4.83±3.78	4.72±4.41	5.24±2.87 <sup>‡</sup>	6.64±4.64 <sup>¶</sup>	0.001
After	5.03±3.72	5.51±4.46	6.63±4.22	7.78±0.4.62	
	NS	NS	NS	NS	

Group P=Placebo, Group C=CLAs, Group E=Vitamin E, Group CE=CLAs+Vitamin E. \*There were no significant baseline differences between groups for all above factors by ANOVA, \*Differences between groups were done by ANCOVA, <sup>a</sup>MMP-3 reduction in group CE compare with group P ( $P=0.002$ ), <sup>b</sup>MMP3 reduction in group E compare with group P ( $P=0.022$ ) was done by *post-hoc* test; Tukey. Statistically significant differences:  $P\leq 0.05$ . After supplementation, <sup>‡</sup>Group E had significantly higher levels than group P ( $P\leq 0.01$ ), <sup>¶</sup>Group CE had significantly higher levels than group P ( $P\leq 0.001$ ). Statistically significant differences between before and after:  $P\leq 0.05$ . CLAs=Conjugated linoleic acids, CCP-A=Citrolinated antibody, MMP3=Matrix metalloproteinase 3, CRP=C-reactive protein, RA=Rheumatoid arthritis, SD=Standard deviation

only in group CE [Table 3] ( $P < 0.05$ ). IL1 $\beta$  increased significantly in group P and E but decreased in group CE ( $P > 0.05$ ). The increase in IL-1 $\beta$  in group E was lower than Placebo. The IL-4 decreased in groups C, E, CE ( $P = 0.03$ ,  $P = 0.07$  and  $P = 0.003$  respectively), but didn't changed significantly in group P. No significant changes were seen in the plasma IL-2 levels in all groups, although increased in group P and decreased in other groups ( $P > 0.05$ ). IL-2/IL-4 increased in all groups with  $P = 0.005$ ,  $P = 0.016$ ,  $P = 0.005$ ,  $P = 0.006$  for group P, CLAs, E and CE respectively, but differences between groups wasn't significant. CCP-A increased in group P

and decreased in group CE significantly ( $P \leq 0.05$ ). The increase in CCP-A in group E was lower than Placebo. MMP-3 increased in group P and decreased in group CE ( $P \leq 0.05$ ), and the differences between group P and group CE was significant ( $P = 0.018$ ) [Tables 3]. The difference between groups for all data except for CCP-A and MMP-3 wasn't significant.

No significant side effects were observed. There were three reports of flatulence (2 in group C and 1 in group CE). It was relieved in two patients by prescribing tablets during meals instead of before meals, but one patient in group C was needed to exclude.

**Table 3:** Levels of some plasma cytokines in patients with active RA before and after 3 months supplementation (mean±SD)

Variables	Group P (n=22)	Group C (n=22)	Group E (n=21)	Group CE (n=22)	P*
IL-1β (pg/ml)					
Before	2.01±0.99	1.91±0.41	1.92±0.42	1.75±0.36 <sup>a</sup>	NS (0.07)
After	3.49±1.15 P=0.004	2.00±0.5 NS*	2.21±0.57 P=0.041	1.73±0.49 NS	
IL-2 (pg/ml)					
Before	28.06±4.37	27.2±5.78	29.13±6.68	27.53±2.75	NS
After	28.68±3.73 NS	27.07±3.75 NS	29.22±6.51 NS	26.53±3.18 NS	
IL-4 (pg/ml)					
Before	19.72±4.99	20.12±5.54	21.12±5.98	18.91±4.13	NS
After	17.61±4.10 P=0.068	16.46±4.23 P=0.03	16.46±3.90 P=0.007	15.66±2.76 P=0.003	
TNF-α (pg/ml)					
Before	14.74±4.52	15.82±6.06	15.43±4.80	13.89±4.18	NS
After	13.61±3.22 NS	13.46±4.77 P=0.01	14.26±3.65 NS	11.48±2.68 P=0.001	
IL-2, IL-4					
Before	1.46±0.22	1.43±0.4	1.46±0.39	1.5±0.27	NS
After	1.68±0.31 P=0.005	1.71±0.36 P=0.016	1.76±0.45 P=0.005	1.72±0.25 P=0.006	

Group P=Placebo, Group C=CLAs, Group E=Vitamin E, Group CE=CLAs+Vitamin E. \*There were no significant baseline differences between groups by ANOVA for all above factors. <sup>a</sup>IL-1β reduction in group CE compare with group P ( $P=0.061$ ) by Tukey and 0.015 by LSD *post-hoc* test, Within each group compare between before and after study was done with pair *t*-tests, ANCOVA tests (controlled variables are age, sex, disease duration). Statistically significant differences between before and after:  $P\leq 0.05$ . CLAs=Conjugated linoleic acids, ANOVA=Analysis of variance, IL=Interleukin, LSD=Least significant difference, TNF-α=Tumor necrosis factor-alpha, SD=Standard deviation, RA=Rheumatoid arthritis, NS=Non significant

## DISCUSSION

Conjugated linoleic acids are groups of fatty acids with the geometrical and positional isomers of linoleic acid. CLAs were previously shown to reduce clinical signs of inflammatory diseases such as type I airway hypersensitivity,<sup>[56]</sup> lupus,<sup>[57]</sup> cancer,<sup>[58]</sup> endotoxin-induced cachexia,<sup>[59]</sup> inflammatory bowel disease,<sup>[60]</sup> and renal failure.<sup>[61]</sup> CLA reduces immune-induced TNF-α<sup>[62,63]</sup> and inducible COX2 expression, key mediators of inflammation in RA. It can also influence cell functions.<sup>[31,63-65]</sup> Based on previous work, it was hypothesized that dietary CLA would have anti-inflammatory effects on animal models of RA.<sup>[63-66]</sup> Some evidence suggests that CLA decreases antigen-induced cytokine production in immune competent cells, modulates the production of cytokines and leukotriene B4, has antioxidant effect,<sup>[63]</sup> and is helpful for reducing symptoms and/or adverse effects of RA.<sup>[28]</sup> CCP-A is a better

index for prediction of erosive RA than ESR, CRP and MMP-3,<sup>[67]</sup> and higher CCP-A concentrations are associated with increased disease activity in RA.<sup>[68]</sup> In our study CCP-A significantly increased in group P ( $P = 0.035$ ) and also E ( $P = 0.055$ ) and decreased in group CE ( $P = 0.034$ ). TNF-α was reduced during our study in treatment groups, but this reduction was significant only in group CE. IL-1β increased in group P ( $P = 0.004$ ) and E ( $P = 0.041$ ) and difference decrease was near significant between group CE, compared with group P ( $P = 0.061$ ). Hence in group P increased CCP-A, MMP-3, and IL-1β, but in group E increased IL-1β and CCP-A (borderline significant, lower than placebo group) and in other groups we hadn't significant increase in those inflammatory parameters. There is evidence for the involvement of T-cells, especially CD4<sup>+</sup> T-cells, in the pathogenesis of RA and is thought to be a Th1 cell-associated disorders rather than Th2<sup>[69]</sup> therefore we measured IL-4 and IL-2 that

**Table 4:** Levels of blood immunity cells in patients with active RA before and after 3 months supplementation (mean±SD)

Variables	Group P (n=22)	Group C (n=22)	Group E (n=21)	Group CE (n=22)	P
WBC (/ $\mu$ L)					
Before	8587.3±2270	7828.6±2465	7724.8±1835	9280.9±2296 <sup>a</sup>	NS*
After	8752.9±2144	8020.9±1633	7993.3±1994	8486.4±2112	
	NS <sup>†</sup>	NS	NS	P=0.038	
Neutrophils (no)					
Before	5099.1±348	1907±4806.8	1311±4800.9	5684.5±1580	NS
After	581±5159.9	4812.5±1519	4762.4±1080	5354.0±1817	
	NS	NS	NS	NS	
Lymphocyte (no)					
Before	2687.8±723 <sup>a</sup>	2439.1±1063	2641.6±730	2776.6±1063b	NS
After	657±3.3107	2610.7±656	2778.1±1496	2518.8±967	
	P=0.006	NS	NS	NS	
Monocytes (no)					
Before	322.8±195.5	356.2±141.2	139.5±295.1	431.5±221.8	NS
After	324.7±94.0	385.0±100.0	290.1±129.1	387.8±158.7	
	NS	NS	NS	NS	
Eosinophils (no)					
Before	84.3±115.6	76.6±71.2	63.8±113.6	85.9±80.4	NS
After	87.6±124.4	94.3±106.6	107.9±134.9	107.8±75.7	
	NS	NS	NS	NS	

Group P=Placebo, Group C=CLAs, Group E=Vitamin E, Group CE=CLAs+Vitamin E. <sup>†</sup>Compare with in each group before with after study by pair *t*-tests, <sup>\*</sup>ANCOVA tests (controlled variables are age, sex, disease duration), <sup>a</sup>Lymphocyte reduction in group CE compare with group P ( $P=0.008$ ), <sup>b</sup>WBC reduction in group CE compare with group P ( $P=0.066$ ). Statistically significant differences between before and after:  $P\leq 0.05$ . CLAs=Conjugated linoleic acids, RA=Rheumatoid arthritis, SD=Standard deviation, WBC=White blood cell, NS=Non significant

respectively secreted by T helper-2 and helper-1 and their ratio. The IL4 decreased in groups C, CE and E ( $P = 0.03$ ,  $P = 0.03$  and  $P = 0.07$  respectively), but did not change in group P significantly ( $P = 0.068$ ). No significant changes were seen in the plasma levels of IL-2 in all groups [Table 5].

Song *et al.* studied the effect of dietary CLA supplementation (3 g/day) on the immune system in healthy humans. CLA supplementation also decreased the levels of the pro-inflammatory cytokines, TNF- $\alpha$  and IL-1 $\beta$  ( $P < 0.05$ ) were similar to our findings, whereas the levels of the anti-inflammatory cytokine IL-10 increased ( $P < 0.05$ ).<sup>[43]</sup> In the Butz *et al.* study on the effect of CLA on collagen antibody-induced arthritis (CIA), CLA-fed mice had arthritic scores 70% that of the CO fed mice.<sup>[66]</sup> Some previous studies on systemic lupus erythematos supported the effects of dietary CLAs. In one study CLA had a beneficial effect in the autoimmune NZB/W F1 mouse, because the cachectic symptom of systemic lupus erythematos was decreased by

dietary CLA and survival days were increased over the control group.<sup>[59]</sup> Several studies reported that CLA can reduce TNF- $\alpha$  levels and TNF- $\alpha$  related cachexia.<sup>[70,71]</sup>

Similar to our study, the mixed CLAs isomers reduce TNF- $\alpha$  level in animals and human primary muscle cells.<sup>[71-73]</sup> CLAs have been shown to reduce pro-inflammatory cytokines, helping to decrease symptoms associated with inflammation.<sup>[33,61-62,74]</sup> However, there are reports that CLAs decreased IL-4 and increased IL-2 and interferon- $\gamma$  (IFN- $\gamma$ ) in splenocytes and T-cells, with no effect on Plasma TNF- $\alpha$  levels or increased it in humans and rats,<sup>[41,75]</sup> which may reflect pro-inflammatory responses by CLAs.<sup>[47,71]</sup> The difference for the effects of CLAs on inflammatory responses may be due to the various isomer used, duration of treatment, tissue specificity and purity.<sup>[63,76,77]</sup> However, the exact mechanism by which CLAs control cytokines are still not clear.<sup>[43]</sup> CLAs may be exerting anti-inflammatory effect through peroxisome proliferator-activated receptors (PPARs). It has

been reported that CLA's effects may be mediated through PPAR- $\alpha$  and PPAR- $\gamma$ .<sup>[62,78]</sup> Activation of PPAR- $\gamma$  inhibits production of inflammatory cytokines, such as TNF- $\alpha$ , IFN- $\gamma$ , IL-1  $\beta$ , IL-6 and IL-8.<sup>[62,77]</sup> Some studies suggest that CLA may affect PPAR- $\gamma$  through nuclear factor- $\kappa$ B.<sup>[63,77-79]</sup>

CLAs are known to inhibit dendritic cell (DC) maturation and have antiinflammatory effects on DC following activation with lipopolysaccharide.<sup>[63]</sup>

CLAs may have direct or indirect anti-inflammatory effect on the activity and expression of COX-2, thus decrease PGE2 production,<sup>[31,80]</sup> we saw a significant reduction in MMP-3 plasma levels in group CE. MMP-3 is an enzyme with abroad substrate specificities and causes joint proteoglycan and other matrix molecules degradation. The imbalance between MMP-3 and its inhibitors leads to joints destruction. MMP-3 increases in the synovial fluid of RA patients and especially in active RA disease and hence it may be a useful biomarker of response to treatment.<sup>[81]</sup>

It has been suggested that nonenzymatic and enzymatic antioxidant systems are impaired in RA; so patients with RA are exposed to oxidant stress.<sup>[10-12]</sup> Several studies show a beneficial effect of antioxidants in RA.<sup>[82,83]</sup> In a case-control study by our group on 59 RA patients and 60 healthy sex and age-matched controls it was shown that in patients with RA Plasma MDA level was significantly higher and plasma concentration of beta-carotene, Vitamin E and Glutathion Reductase activity were significantly lower than healthy controls ( $P < 0.001$ ).<sup>[12]</sup> CLAs may confer antioxidant effect. Arab *et al.* studied the effects of some fatty acids on the redox status and lipid peroxidation of human fibroblasts, but only arachidonic acid and CLA enhanced Glutathion content through  $\gamma$ -Glutamylcysteine ligase induction. CLA was more potent than arachidonic acid in Glutathion synthesis induction.<sup>[84]</sup>

Several studies suggest positive effects of antioxidants such as Vitamin E in RA.<sup>[83,85]</sup>

The cellular responses to Vitamin E are associated with transcriptional and posttranscriptional events. Activation of diacylglycerol kinase and protein phosphatase 2 $\alpha$ , and the inhibition of PKC, COX, lipoxygenase, tyrosine kinase phosphorylation, and cytokine release by Vitamin E are all examples of posttranscriptional regulation.<sup>[86]</sup>

Vitamin E supplementation also resulted in the suppression of pro-inflammatory cytokines such as IL-6 and chemokines such as IL-8 and monocytes chemotactic protein-1 (MCP-1). Vitamin E also modulates cyclooxygenase-2 activity and inhibits thromboxane formation.<sup>[87]</sup> In this study, we used Vitamin E supplementation as an antioxidant, and in combination with CLAs.

The lymphocytes increased in group P ( $P \leq 0.05$ ) and decreased in group CE nonsignificantly.

To the best of our knowledge, the effect of Vitamin E on cytokines in RA patients has not been investigated up to now. Pallest study (1999) in elderly person shows that Vitamin E increases IL4 and IL2 nonsignificantly,<sup>[88]</sup> but alber study) 2003 (on rats shows Vitamin E reduces IL4 and INF $\gamma$  in spleen and lymph nodes.<sup>[40]</sup> In van Tits *et al.* study  $\alpha$ -tocopherol supplementation decreased production of cytokines and superoxide by leukocytes *ex vivo* in both hypertriglyceridemic and normolipidemic persons.<sup>[89]</sup> In Mol *et al.* study was shown that the supplementation of 600 IU/d for four weeks decreased plasma IL-1 $\alpha$ , TNF- $\alpha$ , and IL-1  $\beta$  in smokers but not in diabetic patients.<sup>[10]</sup>

In our study, Vitamin E supplementation for three months reduced IL4 and IL1  $\beta$  significantly. The suggested mechanism may be either directly change in T-cell surface receptors or indirectly effect on macrophages, reduction in PGE2 and H<sub>2</sub>O<sub>2</sub> (product of activated macrophages).<sup>[90,91]</sup> Some studies show the effectiveness of Vitamin E on reducing the pain<sup>[29,85,86]</sup> inflammation and other clinical outcomes that may verify our data.

## CONCLUSIONS

In our study, 3-month co-supplementation with CLA and Vitamin E resulted in a significant reduction of WBC count, MMP-3, and TNF- $\alpha$  in patients with active RA. It seems that CLAs may decrease inflammation in patients with RA. Co-supplementation with Vitamin E could be helpful in increasing the antiinflammatory effects of CLAs on active RA, but further investigations are needed to determine the exact effects of CLAs on inflammation and Immunity in humans.

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