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RESEARCH

# Effects of Selenium Supplementation on the Indices of Disease Activity, Inflammation and Oxidative Stress in Patients with Rheumatoid Arthritis: a Randomized Clinical Trial

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## Abstract

The aim of study was to evaluate the effect of selenium supplementation on disease activity, inflammation, and oxidative stress in patients with rheumatoid arthritis (RA). This study was a randomized double-blind placebo-controlled trial on 59 patients with RA. Participants were randomly divided to receive 200 µg/day of selenium or a placebo for 12 weeks. The disease activity score (DAS.CRP and DAS.ESR), erythrocyte sedimentation rate (ESR), serum levels of C-reactive protein (CRP), fasting blood glucose, lipids, antibodies to cyclic citrullinated protein (anti-CCP), nitric oxide, glutathione, and total antioxidant capacity were assessed. The mean of DAS.CRP and DAS.ESR decreased significantly within both study groups after the intervention. However, the between-group comparisons revealed no significant differences. The CRP levels decreased significantly in the selenium group, and this decrease was near the significance level compared to the placebo ($P = 0.05$). However, after adjusting for baseline values, the observed difference between groups did not remain significant. In addition, the values of ESR and anti-CCP decreased significantly within the selenium group. Although, between-group comparison did not statistically significant, the change in ESR and anti-CCP in the selenium group was small clinically relevant compared to the placebo [the effect size (95% CI) for ESR: 0.38 (− 0.14, 0.89), and for anti-CCP: 0.32 (− 0.2, 0.83)]. Our study showed that selenium caused a small clinically relevant improvement in some RA biomarkers such as ESR and anti-CCP. Future studies that evaluate the effects of novel forms of supplements such as selenium nanoparticles on the clinical symptoms and biomarkers of RA are suggested. Trial Registration: At www.irct.ir as IRCT20190924044869N1 on 2020–06-14.

**Keywords** Selenium · Supplementation · Rheumatoid arthritis · Inflammation · Oxidative stress · Biomarkers

## Introduction

Selenium is an essential element with antioxidant properties that can affect immune and inflammatory responses [1]. The tissue level of selenium depends on its presence in the diet, soil, and geographical distribution of this element [2]. Rice, eggs, tuna, and nuts are some of the food sources that are rich in selenium [3]. This element is found in the form of selenoproteins in the body and plays a role in reducing organic and inorganic peroxides and enhances the metabolism of hydroperoxide during the production of prostaglandins and leukotrienes from arachidonic acid [4–6]. Selenium is an essential co-enzyme of the glutathione peroxidase which is responsible for the detoxification of hydrogen peroxide [7]. In addition to its antioxidant and anti-inflammatory properties, selenium can modulate the differentiation and activation of inflammatory cells by affecting intercellular signals [8–11]. As a result, it is proposed that

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selenium supplementation would have beneficial effects on the prevention and treatment of autoimmune and inflammatory diseases such as rheumatoid arthritis (RA). RA is mainly characterized by the inflammation of small and large joints [12, 13]. The disease destroys the synovial layer leading to deformity and joint pain which in turn reduces a person's function [14, 15]. The prevalence of RA is reported to be between 0.5 and 1% in industrialized countries [16]. In recent years, it has been proven that the risk of cardiovascular disease and atherothrombotic events in patients with RA is higher than healthy people in the community [17–19]. Although the etiology of RA is not fully understood, various factors such as genetics (up to 50%), environmental factors, and vitamin D deficiency are involved [16, 20, 21]. Oxidative stress and various types of reactive oxygen species (ROS) can also play an important role in the pathogenesis of RA [22, 23]. Therefore, selenium, as an anti-oxidant and anti-inflammatory element, seems to have beneficial effects on the treatment of RA. Previous observational studies reported that the patients with RA had significantly lower levels of serum selenium compared to the healthy ones [24–27]. In addition, a promising beneficial effect of selenium supplementation has been found in animal models of RA [28, 29]. However, very limited and old studies investigated the effects of selenium supplementation on RA in clinical trial models [30–32]. Peretz et al. in 1992 showed that supplementation of 200 µg selenium in patients with RA ($n=8$), for 3 months, led to a significant decrease in joints' pain and antigenic score compared to the placebo ($n=7$) [31]. On the other hand, the same investigators conducted a randomized clinical trial in 2001 with more sample size ($n=55$) and reported that selenium supplementation (200 µg/day) did not show clinical benefit on RA [32]. To the best of our knowledge, no study has been published for this purpose since then. Therefore, to renew the evidence, the present randomized clinical trial was performed to investigate the effects of selenium supplementation on the disease activity, inflammatory factors, and oxidative stress biomarkers in patients with RA.

## Method

### Study Participants

Men and women aged between 18 and 75 years and diagnosed with RA were recruited from the Shahid Beheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran. A rheumatologist approved the RA in the study participants if at least four of the following seven criteria of the American College of Rheumatology had been present for at least 6 weeks: morning stiffness; arthritis of three or more joint areas; arthritis of the hands; symmetric arthritis, rheumatoid nodules; positive rheumatoid factors, radiographic, and typical clinical changes in the joints. Another inclusion criterion was that the treatment protocol of RA in participants was unchanged for at least the last two months. Patients were excluded from the study if any of the following conditions existed: (1) having a history of hypothyroidism or hyperthyroidism, and kidney or liver failure; (2) having hypertension before or during the intervention; (3) having the other bone or joint diseases based on the rheumatologist's diagnosis; (4) having an active infection or co-occurrence of another rheumatic disease; (5) failure to adhere to the intervention protocol. Also, the intake of dietary or anti-oxidant supplements, during the last 3 months, was an additional exclusion criterion.

The study detail from Knyazeva et al. was used for the sample size calculation [33]. They examined the influence of selenium on clinical and laboratory parameters in patients with rheumatoid arthritis and subclinical hypothyroid dysfunction. According to their results in terms of the changes in erythrocyte sedimentation rate (ESR), 30 subjects were selected for each arm of our trial to detect a change of 5.5 mm/h in ESR with 80% power and 5% significance. To cover possible dropouts, 15% more subjects were added to the sample size.

The Ethics Committee of Kashan University of Medical Sciences approved the study protocol (Registration No. IR.KAUMS.MEDNT.REC.1398.129). All participants signed the written informed consent to participate in the study. The trial was registered at IRCT.ir (IRCT20190924044869N1).

### Study Design

The present study was a randomized, double-blind placebo-controlled trial with a parallel design. Patients were randomly assigned into two groups. The blocked randomization technique with a 1:1 ratio was used to achieve the balanced group sizes. The sequence of permuted blocks was generated with a computer random number generator. A person, who was not involved in the main process of the trial, packed the supplements and placebos in numbered bottles based on the random list. The bottles were the same in shape and physical properties. The other person, who was also not involved in the trial and not aware of random sequences, assigned the patients to the numbered bottles. Therefore, participants, investigators, and the assessors of the outcomes were unaware of the randomization and allocation to the study groups. The study participants received one capsule containing 200 µg of selenized yeast or one capsule of placebo containing edible starch, daily for 12 weeks. The placebo capsules were similar in shape, color, size, and other physical characteristics to the selenium ones. The adherence to the study protocol was evaluated by counting the unused

capsules which were returned to the researchers. Patients were requested to avoid taking other supplements during the study. In addition, they were contacted twice a month to be reminded of taking supplements and asked about any possible side effects. Also a checklist was prepared that questioned about the use of supplements or medications during the intervention. If the patient had used other antioxidant supplements or new medications during the intervention, he or she was excluded from the final analysis.

### Assessment of Anthropometric Variables and Disease Activity Score of RA

Anthropometric variables including height and weight were measured at the beginning and end of the study. Weight was measured with light clothing and no shoes using a digital scale with a precision of 0.1 kg. Height was measured using a stadiometer in a standing position without shoes with a precision of 0.1 cm. The body mass index (BMI) was calculated using the Quetelet formula: BMI= mass (kg)/height $(m)^2$. At baseline, demographic data and history of diseases, medications, and supplements were obtained from the patients. Subjects were advised not to take any other supplements during the intervention.

To assess disease activity and treatment response of RA in our participants, the disease activity score 28 (DAS 28) criteria were used. DAS 28 considers 28 tender and swollen joint counts, general health (GH; patient assessment of disease activity using a 100-mm visual analog scale (VAS) with 0 = best, 100 = worst), plus levels of an acute phase reactant either ESR (mm/h) or C-reactive protein (CRP) (mg/l)). DAS 28 values were calculated as follows: DAS 28 (CRP) $= 0.56*\sqrt{(TJC28)} + 0.28*\sqrt{(SJC28)} + 0.014*GH + 0.36*\ln(CRP+1) + 0.96$; DAS 28 (ESR) $= 0.56*\sqrt{(TJC28)} + 0.28*\sqrt{(SJC28)} + 0.014*GH + 0.70*\ln(ESR)$,where TJC = tender joint count and SJC = swollen joint count.

### Biochemical Measurements

Blood samples (10 ml) were taken from participants at 8 to 10 a.m. after 12 h of fasting at baseline and after 12 weeks of intervention. About 4 ml of each blood sample was mixed with sodium citrate as an anticoagulant. The remained sample was centrifuged at 3000 rpm to prepare serum. Serum specimens were stored in a freezer at − 70 °C until further assessment.

Fasting blood glucose (FBG) was evaluated using the glucose oxidase method (GOD-PAP, Pars Azmoon Co, Tehran, Iran). High-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), and the low-density lipoprotein cholesterol (LDL-C) were measured via laboratory kits (Pars Azmoon Co, Tehran, Iran) and auto-analyzer (Roche/Hitachi 902). Erythrocyte sedimentation rate (ESR) was evaluated by the Westergren method, ranging from 0 to 140 mm/h. A standardized, upright, elongated tube is used to measure the distance (in millimeters) red blood cells fall to the bottom over 1 h when exposed to gravity. Serum CRP was measured using enhanced turbidimetric immunoassay by laboratory kit (Pars Azmoon Co, Tehran, Iran). In this test, the CRP present in the patient's sample forms a complex with the sensitized antibody against human CRP and causes turbidity. The amount of turbidity created has a direct relationship with the amount of CRP present in the patient's sample. In the present study, CRP levels in the samples were measured against a blank at a wavelength of 340 nm by a spectrophotometer (PD-303UV, APEL Co, Japan).

Antibodies to cyclic citrullinated protein (anti-CCP) were analyzed by enzyme-linked immunosorbent assay (ELISA) (EuroDiagnostica, Sweden). After the completion of the ELISA, we read the yellow color's optical density using an automated spectrophotometer at 450 nm (PD-303UV, APEL Co, Japan). The Griess method was used to measure the serum nitric oxide (NO) [34]. As a first step in this procedure, nitrite was treated with a diazotizing reagent in acidic media. N-Naphthyl-ethylenediamine (NED) was then used as a coupling reagent to form a stable azo compound. Then the purple color of the product was measured at 540 nm by the spectrophotometer. More details about the Griess method are given in the study by Sun et al. [34].

The total antioxidant capacity (TAC) of serum samples was determined by Benzie and Strain methods (ferric reducing antioxidant power (FRAP) assay) [35]. In this assay, 100 µL of serum was mixed with 900 µL of distilled water and 2 mL of the working reagent. After 30 min, the absorbance was measured at 593 nm against the blank reagent. The ascorbic acid (1000 µM) was used as the standard. The result was expressed as µmol/L of ferrous equivalent.

Serum glutathione was quantified using the Beutler method [36]. In this method, distilled water was used to dilute 100 µL of serum sample. Then, 500 µL of the diluted serum was mixed with 2-mL phosphate solution and 250 µL DTNB [5,5-dithiobis (2 nitrobenzoic acid)] reagent. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against a blank (PD-303UV, APEL Co, Japan).

### Primary and Secondary Outcomes

The primary outcomes included (1) changes in DAS-CRP; (2) changes in DAS-ESR; (3) Changes in ESR; (4) changes in the serum levels of CRP. The secondary outcomes included (1) changes in the fasting plasma glucose; (2) changes in the serum levels of glutathione; (3) changes in the total antioxidant capacity; (4) changes in levels of lipid profile; (5) changes in the serum levels of nitric oxide; and (5) changes in the levels of anti-CCP.

## Statistical Analysis

To evaluate the normality of data distribution, the Shapiro–Wilk test was performed. The within-group comparison was done by the paired *t* test (in parametric conditions) or Wilcoxon rank test (in non-parametric conditions). Independent sample *t* test or Mann–Whitney *U* test was utilized for between-group comparisons. The analysis of the covariance (ANCOVA) test was conducted to analyze the differences between the two groups while adjusting for baseline measurements and covariates. In the present study, statistical analyses were conducted by the SPSS software, version 16 (SPSS Inc., Chicago, Ill). Two-sided *P* values $< 0.05$ were statistically significant.

## Results

Out of the 68 patients with active RA that were assigned to the trial groups, nine did not complete the research; five in the selenium group; and four in the placebo group due to unwillingness to continue the study or poor adherence to the research protocol (Fig. 1). Participants were recruited from February 2020 to December 2020. The compliance rate was above 80% in both study groups, and no side effects were reported throughout the research. Table 1 presented the baseline general characteristics of the study groups. There was not any statistically significant difference between the groups regarding gender, smoking, age, BMI, and medical history (Table 1).

Within- and between-group comparisons of the baseline, endpoint, and changes' values for the variables of the severity of RA have been shown in Table 2. Accordingly, the mean values of DAS.CRP and DAS. ESR decreased significantly within both study groups. However, the between-group comparisons revealed no significant differences in this regard.

Table 3 indicates within-group and between-group comparisons of the values of biochemical variables. Serum levels of CRP decreased significantly in the selenium group. In addition, the difference in the mean changes of CRP between the study groups was near the significance level. However, after adjusting for baseline variables, the observed significant difference in CRP between study groups disappeared (Table 3). The estimated marginal mean ± SE of CRP in selenium and placebo group, after adjustment of baseline

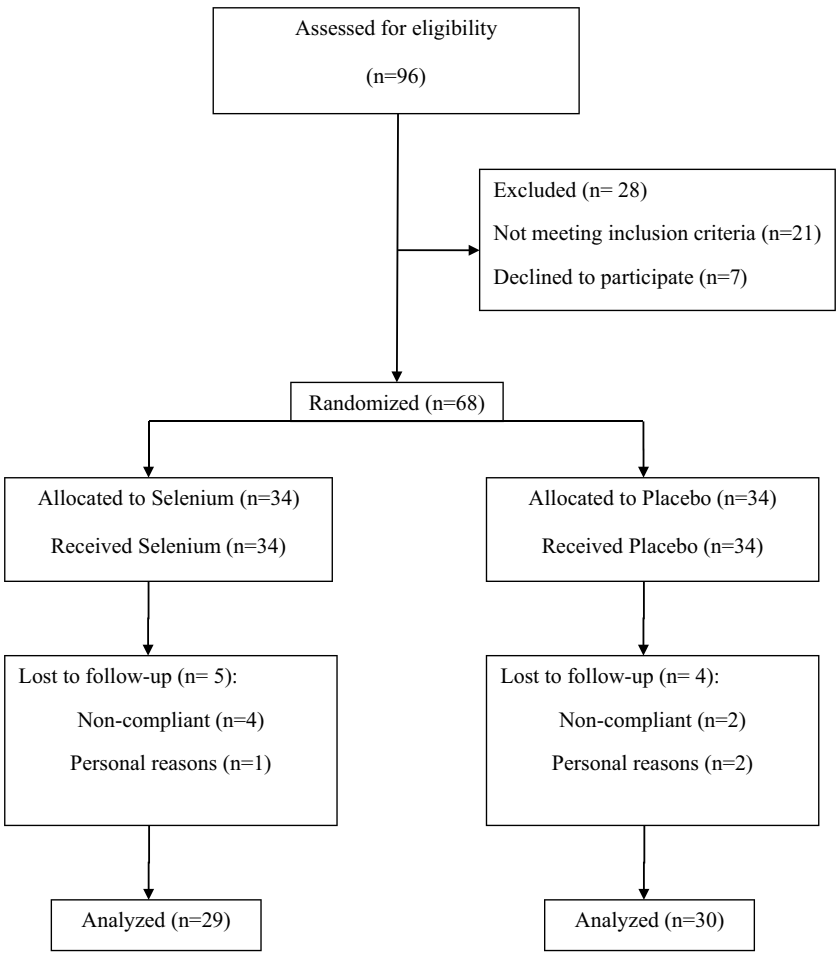


**Fig. 1** Flowchart of patients' recruitment

**Table 1** General characteristics of the study participants

| Characteristics | Selenium $n=29$ | Placebo $n=30$ | $P$ value |
|---|---|---|---|
| Gender (women) (%)[a] | 86.2 | 83.3 | 0.76 |
| Smoker (%)[a] | 3.4 | 3.3 | 0.99 |
| History of diabetes mellitus (%)[a] | 20.7 | 6.7 | 0.14 |
| History of hypertension (%)[a] | 24.1 | 6.7 | 0.08 |
| Ischemic heart disease (%)[a] | 6.9 | 6.7 | 0.97 |
| Age[b] (year) | 50.79 ± 11.31 | 53.30 ± 10.33 | 0.47 |
| BMI[b] ($Kg/m^2$) | 27.85 ± 5.97 | 28.36 ± 5.53 | 0.77 |
| Duration of rheumatoid arthritis[b] (years) | 9.15 ± 9.22 | 10.36 ± 8.64 | 0.31 |

*BMI*, Body mass index

[a]Data is tested by chi-square test or Fisher's exact test

[b]Data is expressed as mean ± standard deviation and tested by two sample $t$ test

**Table 2** Within-group and between-group comparisons of the baseline, endpoint and changes' values for the variables of the severity of rheumatoid arthritis in selenium and placebo groups

| Variables | Selenium group[a] ($n=29$) | Control group[a] ($n=30$) | $P$ value[b] (between) | $P$ value[c] (ANCOVA) |
|---|---|---|---|---|
| VAS (mm) | | | | |
| Baseline | 73.8 ± 15.2 | 66.7 ± 17.7 | 0.131 | |
| Endpoint | 49 ± 20.4 | 43 ± 27.3 | 0.347 | 0.741 |
| Change[d] | − 24.8 ± 21.5 | − 23.7 ± 19.2 | 0.827 | |
| $P$ value (within)[e] | < 0.001[f] | < 0.001[f] | | |
| Number of painful joints | | | | |
| Baseline | 5.7 ± 2.8 | 4.7 ± 2.6 | 0.131[f] | |
| Endpoint | 2.1 ± 2.1 | 2.2 ± 2.4 | 0.292 | 0.324 |
| Change | − 3.6 ± 3 | − 2.4 ± 2.9 | 0.119[f] | |
| $P$ value (within) | < 0.001[f] | < 0.001[f] | | |
| Number of tender joints | | | | |
| Baseline | 3.7 ± 2.8 | 3 ± 3.1 | 0.222 [f] | |
| Endpoint | 1 ± 1.5 | 1.1 ± 2.1 | 0.881[f] | 0.271 |
| Change | − 2.7 ± 2.4 | − 1.9 ± 2.5 | 0.115[f] | |
| $P$ value (within) | < 0.001[f] | < 0.001[f] | | |
| DAS28.CRP | | | | |
| Baseline | 4.5 ± 0.7 | 3.97 ± 1.03 | 0.019 | |
| Endpoint | 3.02 ± 0.91 | 2.78 ± 1.1 | 0.375 | 0.810 |
| Change | − 1.5 ± 0.85 | − 1.19 ± 1.01 | 0.201 | |
| $P$ value (within) | < 0.001 | < 0.001 | | |
| DAS28.ESR | | | | |
| Baseline | 4.8 ± 0.98 | 4.5 ± 0.87 | 0.263 | |
| Endpoint | 3.39 ± 1.08 | 3.13 ± 0.97 | 0.580 [f] | 0.734 |
| Change | − 1.41 ± 1.07 | − 1.39 ± 0.86 | 0.952 | |
| $P$ value (within) | < 0.001[f] | < 0.001 | | |

*ANCOVA*, Analysis of covariance; *DAS*, disease activity score; *VAS*, visual analogue scale

[a]Values are expressed as mean ± standard deviation

[b]$P$ value for comparing the values between the study groups at baseline, at the endpoint and the change from baseline. Two sample $t$ test and Mann–Whitney $U$ test were used for parametric and non-parametric comparisons, respectively

[c]$P$ value for ANCOVA test to determine the significant levels of differences between the two groups post-intervention while adjusting for baseline measurements and covariates

[d] Endpoint values minus the baseline ones

[e]$P$ value for comparing baseline with end point values within each group. Paired sample $t$ test and Wilcoxon paired rank test were used for parametric and non-parametric comparison, respectively

[f]$P$ value obtained from a non-parametric test

**Table 3** Within-group and between-group comparisons of the baseline, endpoint and changes' values for the biochemical variables in selenium and placebo groups

| Variables | Selenium group[a] ($n=29$) | Control group[a] ($n=30$) | $P$ value[b] (between) | $P$ value[c] (ANCOVA) |
|---|---|---|---|---|
| CRP (mg/L) | | | | |
| Baseline | 17.1 ± 13.9 | 12.2 ± 16.1 | 0.075 [f] | |
| Endpoint | 7.9 ± 9 | 8.1 ± 9.6 | 0.682 [f] | 0.516 |
| Change [d] | − 9.2 ± 14.6 | − 4.1 ± 13.2 | 0.05 [f] | |
| $P$ value (within)[e] | 0.001[f] | 0.159[f] | | |
| ESR (mm/h) | | | | |
| Baseline | 34 ± 25.6 | 24 ± 15.7 | 0.179 | |
| Endpoint | 20.3 ± 14.8 | 18.5 ± 14.5 | 0.785 | 0.874 |
| Change | − 13.7 ± 25.3 | − 5.5 ± 17 | 0.375[f] | |
| $P$ value (within) | 0.005[f] | 0.085[f] | | |
| FBG (mg/dl) | | | | |
| Baseline | 113.2 ± 39.1 | 96.5 ± 20 | 0.003 [f] | |
| Endpoint | 105.8 ± 37.5 | 96.1 ± 23 | 0.335 [f] | 0.940 |
| Change | − 7.4 ± 35.9 | − 0.4 ± 14 | 0.041[f] | |
| $P$ value (within) | 0.01[f] | 0.926[f] | | |
| TG (mg/dl) | | | | |
| Baseline | 119.5 ± 49.4 | 144.8 ± 54.9 | 0.026 [f] | |
| Endpoint | 105.8 ± 45 | 130.7 ± 49.7 | 0.030 [f] | 0.211 |
| Change | − 13.7 ± 291 | − 14.1 ± 47.1 | 0.968 | |
| $P$ value (within) | 0.015 | 0.042 | | |
| TC (mg/dl) | | | | |
| Baseline | 185.5 ± 40 | 182.7 ± 50.2 | 0.779 [f] | |
| Endpoint | 176.3 ± 45.3 | 169.2 ± 37 | 0.519 [f] | 0.711 |
| Change | − 9.2 ± 37.4 | − 13.5 ± 52.6 | 0.720 | |
| $P$ value (within) | 0.199 | 0.165 [f] | | |
| LDL (mg/dl) | | | | |
| Baseline | 98.5 ± 32.6 | 92.8 ± 28.5 | 0.478 | |
| Endpoint | 90.7 ± 31.7 | 94.5 ± 32.4 | 0.647 | 0.754 |
| Change | − 7.8 ± 34.2 | 1.7 ± 31.8 | 0.272 [f] | |
| $P$ value (within) | 0.228 | 0.772 | | |
| HDL (mg/dl) | | | | |
| Baseline | 51.2 ± 7.1 | 53.8 ± 11.7 | 0.643 [f] | |
| Endpoint | 50.1 ± 12.3 | 51.4 ± 8.9 | 0.909 [f] | 0.851 |
| Change | − 1.1 ± 11.4 | − 2.4 ± 13.4 | 0.500 | |
| $P$ value (within) | 0.923 | 0.471 | | |
| NO (μmol/L) | | | | |
| Baseline | 220.8 ± 37.2 | 227.4 ± 40.8 | 0.313 [f] | |
| Endpoint | 240.8 ± 31.9 | 241 ± 51.5 | 0.271 [f] | 0.320 |
| Change | 20 ± 35.2 | 13.6 ± 68.8 | 0.650 | |
| $P$ value (within) | 0.007[f] | 0.280 | | |
| Anti CCP (EU/ml) | | | | |
| Baseline | 225.7 ± 199.8 | 163.3 ± 123 | 0.292 | |
| Endpoint | 140.3 ± 141.4 | 131.8 ± 129.5 | 0.915 | 0.773 |
| Change | -85.4 ± 211.5 | -31.5 ± 114.5 | 0.379 | |
| $P$ value (within) | 0.028 [f] | 0.153 [f] | | |
| TAC (mmol/L) | | | | |
| Baseline | 655 ± 111.5 | 665.8 ± 101.2 | 0.699 | |
| Endpoint | 635.7 ± 132 | 691.5 ± 168.1 | 0.161[f] | 0.359 |
| Change | − 19.3 ± 145.1 | 25.7 ± 158 | 0.240 [f] | |
| $P$ value (within) | 0.480 | 0.607[f] | | |

**Table 3** (continued)

| Variables | Selenium group[a] ($n=29$) | Control group[a] ($n=30$) | $P$ value[b] (between) | $P$ value[c] (ANCOVA) |
|---|---|---|---|---|
| GSH (μmol/L) | | | | |
| Baseline | 683.8 ± 161.5 | 737.3 ± 135.5 | 0.062 [f] | |
| Endpoint | 787.9 ± 243.8 | 787.1 ± 173.5 | 0.633 [f] | 0.718 |
| Change | 104.1 ± 254.1 | 49.8 ± 216.6 | 0.580 [f] | |
| $P$ value (within) | 0.06 [f] | 0.280 [f] | | |

*ANCOVA*, Analysis of covariance; *Anti-CCP*, antibodies to cyclic citrullinated protein; *CRP*, C-reactive protein; *ESR*, erythrocyte sedimentation rate; *FBG*, fasting blood glucose; *GSH*, glutathione; *HDL-c*, high density lipoprotein-cholesterol; *LDL-C*, low density lipoprotein cholesterol; *NO*, nitric oxide; *TAC*, total antioxidant capacity; *TC*, total cholesterol; *TG*, triglyceride

[a]Values are expressed as mean ± standard deviation

[b]$P$ value for comparing the values between the study groups at baseline, at the endpoint and the change from baseline. Two sample $t$ test and Mann–Whitney $U$ test were used for parametric and non-parametric comparisons, respectively

[c]$P$ value for ANCOVA test to determine the significant levels of differences between the two groups post-intervention while adjusting for baseline measurements and covariates

[d]Endpoint values minus the baseline ones

[e]$P$ value for comparing baseline with end point values within each group. Paired sample $t$ test and Wilcoxon paired rank test were used for parametric and non-parametric comparison, respectively

[f] $P$ value obtained from a non-parametric test

values, was 7.2 ± 1.6 and 8.7 ± 1.5 mg/L, respectively. Selenium supplementation significantly decreased the serum levels of FBG compared to the placebo. However, this result did not remain significant after adjusting for baseline values (Table 3). The estimated marginal mean ± SE of FBG in selenium and placebo group, after adjustment of baseline values, was 100 ± 4.6 and 101 ± 4.5 mg/dl, respectively.

The serum levels of ESR, anti-CCP, and NO significantly improved within the selenium group; however, the corresponding changes were not significantly different between the study groups. Of note, the change in ESR and anti-CCP in the selenium group was small clinically relevant compared to the placebo [the effect size (95% CI) for ESR: 0.38 (−0.14, 0.89), the effect size (95% CI) for anti-CCP: 0.32 (−0.2, 0.83)]. At the end of the trial, a nearly significant increase in serum GSH was observed in the selenium group, although this increase did not significantly differ from those of the placebo group.

## Discussion

The present study was performed to evaluate the effects of selenium supplementation on disease activity, inflammatory factors, and oxidative stress in patients with RA. The obtained results showed that the clinical markers of RA, such as VAS, number of painful and tender joints, and DAS 28 were similarly and significantly reduced in both groups after selenium and placebo supplementation. However, the between-group comparison of changes regarding the mentioned variables was not significant. This finding was in line with previous studies such as the studies by Peretz et al. and Terp et al. [30, 32]. It seems that in the case of variables, which their evaluation is subjective and perceived by the participants, the placebo effect may be able to mask the effects of selenium supplementation. A similar result was also observed and discussed in the study of Peretz et al. [32]. However, in a clinical trial study conducted in 1992 by Peretz et al., supplementation with 200 μg of selenium caused a significant reduction in pain and joint score compared to the placebo [31]. Systemic inflammation is one of the consequences of RA, which can increase the risk of other chronic diseases such as cardiovascular diseases [37]. Among the important biomarkers of inflammation, we can mention CRP, ESR, and anti-CCP, which also play a diagnostic role in RA [38, 39]. The reduction of these biomarkers can indicate the reduction of RA activity. Previous evidence has shown that selenium can reduce inflammatory mediators by inhibiting nuclear factor kappa-B (NF-kB) expression [40]. In the present study, the CRP levels in the selenium group decreased significantly, and this decrease was close to the significance level when compared to the placebo group. However, after adjusting for baseline values, the observed difference between groups did not remain significant. This finding was very similar to the results of Peretz et al.'s study in which supplementation with 200 μg of selenium caused a significant decrease in CRP level compared to placebo [32]. In the present study, the values of ESR and anti-CCP decreased significantly within the selenium group. Although between-group comparison did not statistically significant, the decrease in ESR and anti-CCP in the selenium group was small clinically relevant compared to the placebo. In Peretz

et al.'s study, although the ESR level decreased within the selenium group, it was not statistically significant [32].

Impaired FBG is more common in patients with RA than in healthy individuals [41]. Increased production of pro-inflammatory factors in RA contributes to insulin resistance and impaired glucose tolerance [42]. On the other hand, the use of glucocorticoid medications in patients with RA can increase blood sugar levels [43]. Considering the antioxidant and anti-inflammatory properties of selenium, it seems that this trace element can be effective in improving glucose metabolism by reducing inflammation. In addition, selenoproteins play an antioxidant role in protecting pancreatic beta cells and consequently regulate glucose metabolism and the expression of genes involved in glycolysis and gluconeogenesis [44]. Therefore, in the present study, the effect of selenium supplementation on FBG in patients with RA was evaluated. After 12 weeks of supplementation, FBG levels in the selenium group decreased significantly compared to the placebo one. However, after adjusting for baseline FPG levels, this difference between the groups was not statistically significant. Controversies have been reported regarding the effect of selenium on FBG in previous studies. Some studies have reported the beneficial effects of selenium on FBG levels, while a limited number of studies have shown the adverse effect [45, 46]. In two recent meta-analysis studies that examined the impacts of selenium supplementation in patients with metabolic diseases, the findings showed that this supplement had no effect on FBG levels [47, 48].

Previous evidence has shown that one of the factors that play a role in the pathophysiology of RA is nitric oxide (NO) [49]. Of note, NO has opposite biological effects in RA, depending on the temporospatial nature of NO action, the level of NO production, intracellular targets of NO, and other environmental and pathophysiological conditions [50]. For instance, the overproduction of NO may be contributed to extensive synovial inflammation in patients with RA [51]. On the other hand, a moderate increase in the levels of NO may promote the apoptosis of the fibroblast-like synoviocytes and prevents angiogenesis in the synovium of patients with RA [50]. In fact, NO can inhibit the mammalian target of rapamycin (mTOR) by phosphorylating the AMP-activated protein kinase (AMPKα) [52]. mTOR is one of the factors affecting the progression of synovitis and structural damage in RA. In this way, by inhibiting mTOR, NO can decrease the synthesis of pro-inflammatory cytokines such as TNFα, IL6, and IL-1β [52]. The only study that investigated the effect of selenium on NO levels in RA was a cell study by Liu et al., who used nanoparticles of selenium [52]. In the present study, NO levels were significantly increased in the selenium group. However, this increase was not significant compared to the placebo group. The findings of a recent meta-analysis study, which was the result of data analysis of 7 clinical trials among the different populations, did not show significant changes in NO levels following selenium supplementation [53]. It seems that more human studies are needed to clarify the effect of selenium on NO production in patients with RA.

Oxidative stress is one of the factors involved in the pathogenesis of RA [54]. During the progression of RA, the inflammatory and hypoxic conditions, created in the synovium, increase the production of reactive oxygen species (ROS) [54]. Increased production of ROS can also increase the severity of synovitis [54]. Selenium is a coenzyme of glutathione peroxidase and plays a role in the activity of this enzyme, which is responsible for neutralizing peroxides. Therefore, it is known as an important factor of the body's antioxidant defense system. In the present study, the effect of selenium supplementation on oxidative stress biomarkers such as TAC and glutathione was investigated. However, no significant difference was observed in the changes of these markers between the selenium and placebo group. Hasani et al. reported that selenium supplementation significantly increased TAC levels, but did not affect glutathione levels in a meta-analysis study that examined the data from 13 clinical trials [55]. However, only 3 included studies investigated the effect of selenium on glutathione [55]. Therefore, more clinical trials, especially in patients with RA, are needed to clarify the effect of selenium on oxidative stress biomarkers.

In the current study, the consumption of 200 µg of selenium was not associated with any side effects. However, some studies have reported evidence of toxicity with high doses of selenium [56]. Therefore, it is necessary to use pharmacological doses of this supplement with caution and with the supervision of the medical staff. Previous evidence has shown that the use of selenium in the form of nanoparticles has lower toxicity and higher bioavailability and, as a result, is more efficient in anti-inflammatory and antioxidant activity [57]. Therefore, investigating the effect of selenium nanoparticles in reducing RA symptoms is suggested in future studies.

In interpreting the findings of the present study, some limitations should be considered. Unfortunately, due to funding limitations, we were not able to measure the serum levels of selenium. Measuring the selenium status with the atomic absorption method has a high cost, and considering the type of the present study and the sample size, this method could not be used. In addition, the dietary intake of selenium was not investigated in the present study. One of the reasons was the lack of reliable information on the selenium content of foods in the national food composition table considering the geographic region. This limitation has been reported by previous publications as a limitation in assessing the selenium intake in different parts of the world [58, 59]. The strength of the present trial was evaluating the effect of selenium supplementation on inflammatory and oxidative stress biomarkers such as anti-CCP, NO, and TAC, which were not investigated in previous

studies. Indeed, using the more informative and precise indicators of RA and oxidative stress, such as the biomarkers of the injury in fatty acids, protein and the nucleic acid, is suggested in future studies to better monitor the effects of selenium supplementation in patients with RA.

## Conclusion

The findings of the present study revealed that the daily consumption of 200 µg of selenium for 12 weeks caused a significant decrease in CRP, ESR, and anti-CCP values in the group receiving selenium. However, these changes were not statistically significant compared to the placebo, although it should be noted that the amount of decrease in ESR and anti-CCP values in the selenium group was small clinically relevant when compared to the placebo. The severity of RA decreased significantly within both study groups, although the difference between the groups was not significant at the end of the study. It is suggested that future studies evaluate the effects of novel forms of supplements such as selenium nanoparticles on the clinical symptoms and biomarkers of RA.

**Author Contribution** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Batool Zamani, Fereshteh Taghvaee, Hossein Akbari, Abbas Mohtashamian, and Nasrin Sharifi. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data Availability** The datasets generated during and/or analyzed during the current study are not publicly available due to the rules and regulations of the Research Center for Biochemistry and Nutrition in Metabolic Diseases at the Kashan University of Medical Science, but are available from the corresponding author on reasonable request.

## Declarations

**Ethics Approval** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Kashan University of Medical Sciences, Kashan, Iran (IR.KAUMS.MEDNT.REC.1398.129).

**Consent to Participate** Informed consent was obtained from all individual participants included in the study.

**Competing Interests** The authors declare no competing interests.

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