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# Zinc supplementation is associated with a reduction in serum markers of inflammation and oxidative stress in adults: A systematic review and meta-analysis of randomized controlled trials

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# Abstract:

**Background:** Zinc (Zn) is a trace metal that is considered to have an impact on chronic inflammation. However, findings of clinical trials have been inconsistent. The present systematic review and meta-analysis aimed to provide a more robust examination of the evidence on the effectiveness of Zn supplements on markers of inflammation and oxidative stress.

**Methods:** A systematic search in PubMed, Scopus, Web of Science and Cochrane Library was undertaken to identify relevant randomized controlled trials (RCTs) assessing the impact of Zn on inflammation and oxidative stress until 17 August 2020. We applied a random-effects method to obtain effect sizes (ES) and 95 % confidence intervals (CIs). Meta-regression was used to detect the potential source of between-study heterogeneity.

**Results:** Twenty-one eligible RCTs comprising 1321 participants were included in the meta-analysis. In comparison with the control groups, serum C-reactive protein (CRP) (ES = -0.92 mg/L, 95 % CI = [-1.36, -0.48], $P < 0.001$, $I^2 = 90.2$ %), tumor necrosis factor-alpha (TNF-α) (ES = -0.49 pg/mL, 95 % CI = [-084, -0.14], $P = 0.006$, $I^2 = 34.6$ %) and malondialdehyde (MDA) (ES = -0.42, 95 % CI = [-083, -0.01], $P = 0.04$, $I^2 = 76.1$ %) were significantly reduced in the groups receiving Zn. Serum interleukin 6 (ES = -1.02 pg/mL, 95 % CI = [-2.06, 0.02], $P = 0.05$, $I^2 = 92.3$ %) was marginally reduced following Zn supplementation. Moreover, treatment duration was found as the source of inter-study heterogeneity.

**Conclusion:** This meta-analysis suggests that Zn supplements reduce serum concentrations of markers of inflammation and oxidation: CRP, TNF-α and MDA.


**Keywords:** Zinc, Inflammation, Oxidative stress, Review, Meta-analysis

# Introduction:

Prasad et al. first noted the importance of zinc (Zn) on human health in the 1960s. Zn is a nutritionally crucial trace element and is the second most plentiful trace metal in the human body after iron (1). Zn has been reported to attenuate chronic inflammatory responses through reduction of serum inflammatory cytokines. It is also reported to reduce oxidative stress by its role as a cofactor of antioxidant enzymes and as an enzyme catalyst, participating in the metabolism of lipids, carbohydrates, and proteins (2). Zn is one of the key elements involved in the activities of more than 300 enzymes and 2800 macromolecules (3). The total Zn content in the average human body has been estimated to be 2–4 g, with a normal plasma concentration of 12–16 µmol/L (4).

Zn status appears to have an impact on many metabolic and chronic diseases, which include cancer (esophageal, breast cancer, lung carcinoma), neurodegenerative diseases, and diabetes. There is also a strong association between Zn deficiency and susceptibility to various infectious diseases such as tuberculosis, measles, HIV, malaria, and pneumonia (5). The $Zn^{2+}$ ion has a vital function in the differentiation, apoptosis, and cell proliferation. In addition, it is involved in metabolism, DNA synthesis, reproduction, and neurophysiological processes. Studies have demonstrated that Zn preserves DNA integrity and its deficiency can inhibit the function of the Zn-dependent proteins participating in the response to DNA damage (6).

Inflammatory processes involve several pathways. Modulation of these pathways is important to provide an adequate response to various stimuli such as free radicals, stress, cytokines, oxidized low-density lipoprotein-cholesterol (LDL) or bacterial/viral pathogens. The nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) is one of the key inflammatory mediators that impacts cellular apoptosis, proliferation and adhesion, tissue remodeling, innate and adaptive immune reactions, inflammatory processes, and cellular stress reactions. NFκB, regulates the expression of proinflammatory cytokines (e.g., IL-6, IL-1b, IL-8, MCP-1, and TNF-α), chemokines, acute phase proteins (fibrinogen and CRP), matrix metalloproteinases (MMPs), adhesion molecules and growth factors (7, 8). Zn status determines the activation of NFκB activation is enhanced or inhibited (9). These data illustrate the impact of Zn on normal metabolism, inflammation and oxidative stress, indicating that Zn supplementation can have important effects on endocrine outcomes, inflammatory biomarkers, and oxidative stress. Several

studies have assessed the effects of Zn supplementation on inflammatory markers and oxidative stress. However, randomized controlled trials (RCTs) have been inconsistent. Moreover, to date, no systematic review, nor meta-analysis have been published specifically on the effects of Zn supplementation on inflammation and oxidative stress in adults. In the present study, a systematic review and meta-analysis of RCTs was conducted to summarize the evidence on the effects of Zn supplementation on inflammatory mediators and oxidative stress in subjects over the age of 18 years.

# Methods:

We designed our study according to the *Preferred Reporting Items for Systematic reviews and Meta-Analyses* (PRISMA) guidelines (10).

## *Search strategy:*

We conducted our systematic search of the following online databases: PubMed, Scopus, Web of Science and Cochrane Library to identify any relevant clinical trial published up to 17 August 2020 and investigated the efficacy of Zn supplementation on inflammatory meditators and oxidative stress. Two authors gathered the primary search terms independently and then the corresponding author reviewed and improved them. At end, our search strategy was contained of the combination of terms, which searched in titles and abstracts and shown in *supplementary data*. To supplement our search process and to avoid missing any relevant article, reference lists of related reviews, *Google Scholar* and *ClinicalTrials.gov* were hand-scanned by two authors, independently and double-checked by the corresponding author.

## *Inclusion and exclusion criteria:*

At the initial stage, we selected any parallel randomized controlled trials (RCTs) reporting the effects of Zn supplementation on at least one of our specified outcomes (e.g. CRP, TNF-α, IL-6 and MDA). At next, we excluded any study if it was dissertation, brief reports, editorials, reviews, letters, and non-randomized, non-English language, animal- or observational-designed. Then, studies which were conducted on children or adolescents (< 18 years old), reported insufficient data (e.g. for calculating standard deviation (SD), standard error (SE), 95 % confidence intervals (CI) or interquartile (IQR)) or non-extractable data were omitted. Finally, studies were checked for the use of an appropriate control and intervention group. We excluded studies if their control

group was not clinically comparable with the intervention, and therefore inclusion would have biased the results. These steps were undertaken by two authors with attention to both clinical and statistical views and cross-checked by corresponding author. Given any doubts about the data, we at first tried to resolve them by group discussion. If there was a disagreement, or key information were ambiguous, we contacted corresponding authors of the relevant articles.

*Data extraction:*

After selecting eligible articles, two authors extracted the following data: first authors last name, corresponding author's e-mail, publication year, country, population, subject's characteristics, intervention and control type, dose and type of supplement, treatment duration, sample size, methodological quality, mean change of interested outcome from baseline at the end of trial, related SD, SE, 95% CI or IQR. In the cases that reported SE, 95% CI or IQR, appropriate formulas were used to obtain the SD (11). Units of interested outcomes were converted to the globally most commons. Given the inconsistencies in the converted units, we saved them as each unit reported in the related article. When the data were presented as a graph, WebPlotDigitizer (*https://automeris.io/WebPlotDigitizer/*) was used to extract mean change and related SD. Methodological quality appraisal was performed by two authors based on Cochrane Collaboration Tools (12), which contained domains such as selection bias, performance bias, detection bias, attrition bias, reporting bias and other sources of bias (e.g. bias of study design, trial stopped early, extreme baseline imbalance and fraudulent). Each domain scored by "Low", "High" or "Unclear" risk of bias. The corresponding author re-checked these analyses.

*Statistical analysis:*

Effect sizes (ES) and corresponding 95% CIs were obtained applying a random-effects model (13). As suggested by the Cochrane guidelines (14), standardized mean difference (SMD) was used as summary measure, which is applicable for interpretation as the ES. We did $I^2$ test to determine the among-study heterogeneity, which was considered as moderate to high with values greater than 50 % (13, 15). Given the presence of high inter-study heterogeneity, a random-effects meta-regression was used to determine if the mean age of the treatment group, dose of Zn or intervention duration could be as its potential sources (13). Subgroup analysis was run for any outcome with more than ten effect sizes based on region, mean age, type of Zn supplement and duration. Moreover, to assess the dependency of pooled results from each trials, we undertook a sensitivity analysis (13).

Egger's and Begg's tests were used to assess the publication bias in significant results (16). If potential publication bias was identified ($P < 0.05$), we applied a "trim and fill" approach to adjust the analysis (17). Net changes were calculated using following procedure: (mean-post – mean-pre). Also, ($\sqrt{([(SD\ pre)^2 + (SD\ post)^2] - [2r \times SD\ pre \times SD\ post])}$) was used to obtain SD for changes, assuming coefficient correlation (r) = 0.5, as it is a conservative estimate for an expected range of 0-1 (18). SE or IQR, SD were calculated by using the following formulas, respectively: SD = SE $\times \sqrt{n}$, SD = IQR / 1.35 (19). Two authors performed the analyses followed by a final revision by the corresponding author. We used *Stata v.13* to analyze all data. *P* values $< 0.05$ regarded the statistical significance.

## Results:

*Systematic review:*

A total of 8692 articles were identified by searching the online databases **(Figure 1).** Duplicate records (n = 3339) were excluded, and the remaining 5353 citations were assessed by screening the titles and abstracts, and this resulted in excluding another 5300 records. A total of 53 full text articles remained for eligibility assessment, of which 32 were excluded for being ineligible. Finally, 21 full-text papers were used for the meta-analysis, which resulted in the estimation of 23 separate effect sizes. The selected papers were published between 2007 and 2020 **(Table 1)**. Of the papers, 16 studies were conducted in Asia (20-35), four in America (36-39), and one in Europe (40). The mean age of subjects in the intervention groups ranged from 20.7 to 75.1 years, and a total of 1321 participants were enrolled into the included studies. Minimum and maximum dosage of Zn were 30 and 528 mg / day, respectively. Gluconate (23, 25, 30, 33, 35-37, 40) and sulfate (20-22, 24, 26-29, 31, 32, 34, 38, 39) were used as the formulation of supplemental Zn in 8 and 13 studies, respectively. Controls received placebo in most of the studies (9, 20-22, 25-36, 38-40). Pakasi et al, reported two effect sizes for having two control groups receiving placebo and vitamin A (21). In addition, in the study of Khan et al, control subjects received oral hypoglycemic agents (24), and in another study by Guo et al. no placebo was used (23). Treatment duration lasted between 2-48 weeks. The results of quality assessment are shown in **Table 2**.

*Meta-analysis:*

Pooling trial data, to examine the effects of Zn supplementation, showed a significant reduction in serum concentration of CRP (ES = -0.92 mg/L, 95 % CI = [-1.36, -0.48], $P < 0.001$, $I^2 = 90.2$ %),

TNF-α (ES = -0.49 pg/mL, 95 % CI = [-084, -0.14], $P$ = 0.006, $I^2$ = 34.6 %) and MDA (ES = -0.42, 95 % CI = [-083, -0.01], $P$ = 0.04, $I^2$ = 76.1 %) in comparison with controls **(Figure 2).**

On the other hand, serum IL-6 (ES = -1.02 pg/mL, 95 % CI = [-2.06, 0.02], $P$ = 0.05, $I^2$ = 92.3 %) was marginally reduced for the groups receiving Zn supplement, compared to controls **(Figure 2).**

*Meta-regression:*

As inter-trial heterogeneity was high in the assessment of the effects on serum CRP, IL-6 and MDA, we used a random-effects meta-regression by age, dose and duration to assess the potential sources of heterogeneity. For serum CRP and IL-6, there was a non significant correlation with the potential moderators. However, serum MDA was significantly correlated with the treatment duration (coefficient = 0.05, 95% CI = [0.001, 0.010], $P$ = 0.04).

*Subgroup analysis:*

**Table 3** outlines the details of subgroup analysis, which categorized based on region, age, type of Zn supplement and duration. Zn supplementation did not significantly affect serum CRP when treatment lasts more than 20 weeks ($P$ = 0.21). In addition, we observed the greater clinical effect of Zn sulfate (ES = -1.14 mg/L) than Zn gluconate (ES = -0.51 mg/L).

*Sensitivity analysis:*

The sensitivity analysis was applied using "one-study-removed" strategy to assess the impact of each study on the effect size. The results of sensitivity analysis indicated that the pooled results of interested outcomes were not sensitive to each study.

*Publication bias:*

Regarding the effects of Zn on serum CRP, both Egger's ($P$ = 0.002) and Begg's ($P$ = 0.006) regression tests indicated a significant publication bias. Hence, we conducted a "trim and fill" analysis to resolve this, and this did not affect the results, suggesting that the publication bias did not significantly affect the results. Moreover, there was no significant publication bias for either TNF-α (Egger = 0.68, Begg = 0.62) or MDA (Egger = 0.37, Begg = 0.32).

# Discussion:

To the best of our knowledge, the present study is the most recent systematic review and meta-analysis specifically on the effects of Zn supplementation on inflammatory markers and oxidative stress in adults.

Our findings indicated that the consumption of Zn significantly reduced serum concentration of CRP, TNF-α and MDA in comparison with controls. This has been shown in other studies. Karamali et al., demonstrated that taking Zn supplements for 6 weeks in women with gestational diabetes resulted in a significant reduction in serum CRP concentrations (30). In addition, Khazdouz et al. (32) reported that inflammatory markers were significantly lower in the Zn-supplemented group (120 mg Zn *via* a nasogastric tube for 15 days) compared to the placebo group among patients with severe head trauma, which is consistent with the study conducted by Khorsandi et al.,(34) in obese subjects. The referred study found that Zn supplementation (30 mg/day for 15 weeks) with a restricted calorie diet (~ 300 kcal lower than the estimated energy requirement) reduced CRP levels compared to the control group. Jamilian et al., found that patients who received Zn supplements had significantly lower MDA levels compared with the placebo. However, no significant effect of Zn supplementation was observed on inflammatory cytokines and biomarkers of oxidative stress (29). Likewise, another study by Dias et al. in patients with atherosclerosis demonstrated that Zn supplements (30 mg/day) and rosuvastatin had no impact on hs-CRP concentration after 4 months (41). The discrepancy of the results in these RCTs may be due to several confounding variables, which include: study population size, Zn intervention dose, duration of supplementation and baseline serum CRP level. The mechanism by which Zn has its anti-inflammatory effect is likely to be mediated by NF-κB reduction, which is regulated by the peroxisome proliferator-activated receptor (PPAR-α) and the A20 protein signaling pathway, leading to a decrease in serum concentrations of inflammatory markers including CRP and TNF-α (42). Zn supplements have an influence on immune cell function, such as macrophages, monocytes, neutrophils, natural killer cells, and T and B cells, which play a significant role in the response to inflammation (37, 43). Kim et al., (25) showed that Zn supplementation markedly decreased the serum levels of IL-6 and hs-CRP after 8 weeks in obese women. Nonetheless, our results indicate that serum IL-6 was marginally reduced in the group receiving Zn supplement, compared to controls. It was reported that cytokines such as IL-1β , IL-6, IL-2 and TNF-α are affected in a dose-dependent way in Zn deficiency (44). In addition, in subgroup analysis we found that Zn supplementation changed the concentration of serum CRP in studies that lasted less than

20 weeks. So based on our results, long-term use of Zn may not be associated with reduced inflammation.

Age-related Zn deficiency can play an important role in age-related immune function dysregulation, and can contribute to age-related inflammation and associated morbidities (45, 46). Recent studies suggest that intracellular Zn homeostasis is involved in signaling in immune cells, and cellular Zn control in these immune cells is mediated by alterations in the expression of different Zn transporters (47, 48). Among the studies included in this meta-analysis, there were only two studies that were performed on the elderly, which were conducted in the United States and Italy. The first study conducted by Venneria et al. (40) provided two effect sizes, in which ones received placebo and other groups received 15 mg/day and 30 mg/day of Zn for 6 months among elderly Italian population. Their results did not show significant changes in serum MDA levels with respect to baseline values. However, Bao et al., (37) reported a reduction in serum hs-CRP, IL-6 and MDA among elderly American subjects as result of Zn supplementation (45 mg Zn / d) after 6 months. Although Zn is known to function as an anti-inflammatory agent, the specific mechanisms that link Zn, age and inflammation remain unclear to date .Therefore, further studies are required to determine causes of the link between Zn deficiency and inflammation in the elderly (49).

The results of subgroup analyses further revealed that the mean difference of variables in the Zn sulfate group showed a greater decrease than the Zn gluconate group. Therefore, it can be concluded that Zn sulfate is clinically more effective in reducing inflammation than Zn gluconate based on our results. It is noteworthy that, Zn sulfate preserved epithelial and tissue integrity by boosting cell development, suppressing apoptosis and acting as an antioxidant that protects against free radical damage during inflammatory process (50). Although, Zhang et al., demonstrated that Zn sulfate and Zn gluconate had equivalent bioavailability based on plasma and tissues Zn levels in rats (51). Moreover, from the limited evidence released on the absorption of Zn from supplements fed to humans, it appears that Zn sulfate and Zn gluconate are absorbed to a similar size (52). To the best of our knowledge, there was no study comparing these supplements in terms of clinical efficacy. Also, due to the higher cost of Zn gluconate than Zn sulfate (53), it could be suggested that future clinical studies consider this issue.

***Strength and weakness:***

In this study, we have comprehensively reviewed the RCTs that have investigated the effects of Zn supplementation on inflammatory mediators and oxidative stress in subjects over the age of 18 years. To our knowledge, except one previous meta-analysis by Mousavi et al. (54) on the effect of Zn supplementation on CRP, no other systematic review or meta-analysis has been published specifically on the effects of Zn supplementation on other clinical inflammatory mediators and oxidative stress in adults. In addition, compared with the mentioned meta-analysis (54), we have included additional studies on CRP (21, 30-32, 34, 35) and inflammatory factors such as TNF-α and IL-6. Moreover, MDA was included in the current study to evaluate the status of oxidative stress. We also applied meta-regression, which led to detecting treatment duration as a source of inter-study heterogeneity. However, the current meta-analysis has some limitations. First, participants were not restricted to the special disease, and because of the variety of these conditions, we were not able to conduct sub-analysis. Second, we did not register our study protocol on *PROSPERO* registry system due to the delay in processing the submitted protocols for studies outside the UK. Third, the number of included studies was not sufficiently large to be allow robust subgroup analyses on outcomes with less than ten effect sizes (55). Fourth, the skewed distribution of CRP might have introduced some error to the analysis, which calls for future attempts with individual patient data. Finally, one issue that could affect the between-study heterogeneity is that the serum Zn concentration may have a circadian alteration (higher in the morning and lower in the afternoon time) (56). In order to clarify the relationship between Zn concentration and disease progression, it is suggested that more studies are needed to better define the mean Zn concentration in healthy and unhealthy populations from different geographical regions.

## *Conclusion:*

In conclusion, the present meta-analysis revealed that Zn supplementation significantly reduced serum CRP, TNF-α, and MDA levels. However, IL-6 was marginally reduced in the group receiving Zn as supplement, compared to controls. Further RCTs with larger sample are required to determine the effective dose and duration of Zn supplementation on inflammatory and oxidative stress markers in different demographic groups.

## *Author contribution:*

RH and MJ contributed to study design and concept. RH, MAM and MJ contributed to literature search, data extraction, and analysis. RH, GAF, AS and MJ contributed to drafting and reviewing the final manuscript. All authors read and approved the paper for publication.

*Ethics approval and consent to participate:*

Not applicable.

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**Table 1.** Main demographic characteristics of the included RCTs

| First author | Publication year | Population | Country | Mean age | Dose (mg / day) | Type of supplement | Control group | Duration (Weeks) | Sample size |
|---|---|---|---|---|---|---|---|---|---|
| **Prasad et al.** | 2007 | Healthy elderly | USA | 32 | 45 | Zn gluconate | Placebo | 48 | 49 |
| **Rashidi et al.** | 2009 | Hemodialysis patients | Iran | 56.4 | 220 | Zn sulfate | Placebo | 6 | 55 |
| **Bao et al.** | 2010 | Healthy elderly | USA | 65 | 45 | Zn gluconate | Placebo | 24 | 40 |
| **Pakasi 1 et al.** | 2010 | Severely malnourished pulmonary tuberculosis patients | Indonesia | 30.9 | 30 | Zn sulfate | Placebo | 24 | 140 |
| **Pakasi 2 et al.** | 2010 | Severely malnourished pulmonary tuberculosis patients | Indonesia | 30.1 | 30 | Zn sulfate + Vitamin A | Vitamin A | 24 | 115 |
| **Pourteymour Fard et al.** | 2011 | PCOS | Iran | 27.17 | 50 | Zn sulfate | Placebo | 8 | 60 |
| **Velázquez-Pérez et al.** | 2011 | Spinocerebellar Ataxia type 2 | Cuba | 42.35 | 50 | Zn sulfate | Placebo | 24 | 33 |
| **Gou et al.** | 2013 | Hemodialysis patients | China | 59 | 78 | Zn gluconate | Nothing | 8 | 65 |
| **Khan et al.** | 2013 | Type-2 diabetic patients with microalbuminuria | India | 56.3 | 50 | Zn sulfate + Oral hypoglycemic agents | Oral hypoglycemic agents | 12 | 44 |
| **Kim et al.** | 2014 | Young Obese Women | Korea | 20.7 | 30 | Zn gluconate | Placebo | 8 | 40 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Meksawan et al.** | 2014 | Type 2 DM and metabolic syndrome | Thailand | 58.75 | 132 | Zn sulfate | Placebo | 8 | 17 |
| **Ranjbar et al.** | 2014 | Depressed outpatient | Iran | 37 | 110 | Zn sulfate | Placebo | 12 | 44 |
| **Venneria 1 et al.** | 2014 | Healthy elderly | Italy | 74.5 | 15 | Zn gluconate | Placebo | 24 | 73 |
| **Venneria 2 et al.** | 2014 | Healthy elderly | Italy | 75.1 | 30 | Zn gluconate | Placebo | 24 | 71 |
| **Karamali et al.** | 2015 | Gestational diabetes | Iran | 34 | 233 | Zn gluconate | Placebo | 6 | 50 |
| **Islam et al.** | 2016 | Pre-diabetics | Bangladesh | 42.1 | 30 | Zn sulfate | Placebo | 24 | 55 |
| **Jamilian et al.** | 2016 | PCOS | Iran | 34 | 220 | Zn sulfate | Placebo | 8 | 48 |
| **Ribeiro et al.** | 2016 | Colorectal cancer | Brazil | 62.5 | 308 | Zn sulfate | Placebo | 16 | 24 |
| **Momen-Heravi et al.** | 2017 | Patients with diabetic foot ulcer | Iran | 58.3 | 220 | Zn sulfate | Placebo | 12 | 60 |
| **Khazdouz et al.** | 2018 | Severe head trauma | Iran | 32 | 528 | Zn sulfate | Placebo | 2 | 94 |
| **Roshanravan et al.** | 2018 | Pregnant women with impaired glucose tolerance | Iran | 29.45 | 30 | Zn gluconate | Placebo | 8 | 44 |
| **Khorsandi et al.** | 2019 | Obese adults | Iran | 35.63 | 30 | Zn sulfate | Placebo | 15 | 40 |
| **Jafari et al.** | 2020 | Premenstrual Syndrome | Iran | 23.04 | 30 | Zn gluconate | Placebo | 12 | 60 |

**Table 2.** Methodological quality assessment based on authors' Cochrane Collaboration Tool

| Study | Sequence generation | Allocation concealment | Blinding | Blinding of outcome assessment | Incomplete outcome data | Selective reporting | Other bias |
|---|---|---|---|---|---|---|---|
| Prasad | + | + | + | + | + | + | + |
| Rashidi | + | + | + | + | + | + | + |
| Bao | + | + | + | + | + | + | + |
| Pakasi | + | + | + | + | + | + | + |
| Pourteymour Fard | + | + | + | + | + | + | + |
| Velázquez-Pérez | + | + | + | + | + | + | + |
| Guo | + | + | ? | ? | + | + | + |
| Khan | + | + | ? | ? | + | + | + |
| Kim | + | + | + | + | + | + | + |
| Meksawan | + | + | + | + | + | + | + |
| Ranjbar | + | + | + | + | + | + | + |
| Venneria | + | + | ? | ? | + | + | + |
| Karamali | + | + | + | + | + | + | + |
| Islam | + | + | + | + | + | + | + |
| Jamilian | + | + | + | + | + | + | + |
| Ribeiro | + | + | + | + | + | + | + |
| Momen-Heravi | + | + | + | + | + | + | + |
| Khazdouz | + | + | + | + | + | + | + |
| Roshanravan | + | + | + | + | + | + | + |
| Khorsandi | + | + | + | + | + | + | + |
| Jafari | + | + | + | + | + | + | + |

+ : Low risk, − : High risk and ? : Unclear

**Table 3.** Subgroup analysis regarding the effects of Zn supplementation on serum CRP by region, age, type of Zn and duration

| Subgroup | | Number of trials | ES (95 % CI) | *P* | $I^2$ |
|---|---|---|---|---|---|
| *CRP* | | | | | |
| **Region** | Asia | 14 | -0.94 (-1.41, -0.47) | < 0.001 | 90.9 % |
| | America | 1 | - | - | - |
| **Age** | More than 40 | 6 | -1.09 (-1.59, -0.59) | < 0.001 | 77.1 % |
| | Less than 40 | 9 | -0.82 (-1.44, -0.20) | 0.01 | 92.6 % |
| **Type of Zn supplement** | Zn sulfate | 9 | -1.14 (-1.76, -0.52) | < 0.001 | 93.0 % |
| | Zn gluconate | 5 | -0.51 (0.95, -0.08) | 0.02 | 65.7 % |
| **Duration** | More than 20 weeks | 5 | -0.27 (-0.69, 0.16) | 0.21 | 71.9 % |
| | Less than 20 weeks | 9 | -1.16 (-1.71, -0.62) | < 0.001 | 89.4 % |
| ES: effect size, CI: confidence intervals, CRP: C-reactive protein | | | | | |

# Figure legends:

**Figure 1.** The process of study selection

**Figure 2.** The effects of Zn supplementation on serum CRP (A), TNFα (B), IL-6 (C) and MDA (D); CRP: C-reactive protein; TNF-α: Tumor necrosis factor-alpha; MDA: Malondialdehyde