Clinical effectiveness of zinc supplementation on the biomarkers of oxidative stress: a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Background: Despite equivocal reports in the literature, no study has summarized the effect of zinc supplementation on oxidative stress. Therefore, this systematic review and meta-analysis was conducted to examine the effect of zinc supplementation on oxidative stress biomarkers.

Methods: Systematic searches were done using the PubMed/Medline, Scopus, and Google Scholar databases, up to April 2020. All RCTs assessed the effect of oral zinc supplementation on serum Malondialdehyde (MDA), Total Antioxidant Capacity (TAC), Glutathione (GSH), and Nitric oxide (NO) levels, were included. For each variable, mean differences and standard deviations (SDs) were combined using the random-effects model, and the fractional polynomial model was used to implement the dose-response analysis.

Results: Overall, 10 RCTs were included. The pooled analysis of data showed zinc supplementation significantly reduced MDA levels (WMD: -0.42 μmol/L; 95% CI: -0.71 to -0.13) and increased serum TAC (WMD: 225.96 mmol/L; 95% CI: 68.42 to 383.5) and GSH levels (WMD: 49.99 μmol/L; 95% CI: 2.25 t 97.73), compared to the placebo group. However, no significant changes were seen in NO levels following zinc supplementation (WMD: -1.66 μmol/L; 95% CI: -5.89 to 2.57). Dose-response analysis showed a significant non-linear relationship only between zinc supplementation dosage and serum levels of MDA ($P < 0.01$).

Conclusions: The current study showed a significant reduction in MDA and a significant increase in TAC and GSH levels by the zinc supplementation. However, we did not detect significant changes in NO levels. Thus, further studies are required to confirm the veracity of our findings.

KEYWORDS: Zinc; Antioxidants; Oxidative stress; Malondialdehyde; Reactive Oxygen Species

INTRODUCTION

Oxidative stress parameters are involved in the natural development of multiple diseases, such as cancer, Alzheimer's disease, and myocardial infarction [1, 2]. These biomarkers represent the imbalance amongst reactive oxygen species (ROS) and biological components to detoxify metabolic wastes as well as to address potential cell injury [3, 4]. An overproduction and activity of oxidative stress compounds have been related to a series of biomolecules impairments, which might possibly lead to programmed cell death [5]. Therefore, oxidative stress might result in rupture of normal cellular physiology and affect cell signalization [6]. Malondialdehyde (MDA), total antioxidant capacity (TAC), glutathione (GSH), and nitric oxide (NO) are important markers of oxidative stress and has been described as benchmarks for several chronic pathologies [7, 8].

Various agents influence the balance of oxidants/antioxidants in the body [9]. It has been identified that nutrition and diet are effective on oxidative stress; which several dietary factors are considered as oxidants or antioxidants [10]. Recent studies have evaluated the function of multiple minerals with antioxidant features [11]. Zinc itself, frequently administered as zinc sulfate or zinc gluconate, has demonstrated effective results related to gastrointestinal functionality, mainly due to restorage of mucosal integrity and enterocyte enzyme activity [12]. In addition, zinc supplementation has been described to act as cell protector against oxidative species and contributor to the immune system [13, 14]. Earlier systematic reviews and meta-analyses have also suggested zinc supplementation may have a beneficial effect on serum levels of LDL cholesterol, triglycerides, total cholesterol, insulin resistance, C-reactive protein concentrations, and systolic blood pressure [15-18]. Thus, considering its biological properties, understanding zinc's effectiveness and harmful features are worthwhile analysis that evidence-based medicine studies should give attention.

Over the last number of years, several publications have established controversies on the efficacy and influence of zinc supplementation in antioxidant profile. Several studies demonstrated beneficial effects of zinc supplementation on antioxidant status [19, 20], while others did not find such favorable effects [21, 22]. Such equivocality may have occurred because of varying study designs, dosage, and time of follow-up, or diverse measurement tools. In addition, to our knowledge, no systematic review and meta-analysis has analyzed the effect of zinc supplementation on oxidative stress parameters. In order to elucidate these aforementioned shortcomings, this study sought to summarize all published randomized controlled trials that evaluated the effects of zinc administration on oxidative stress biomarkers (MDA, TAC, GSH, and NO).

METHODS

Th design, conduct, and reporting of the present systematic review and meta-analysis adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [23].

Literature search

Comprehensive computerized systematic searches were implemented throughout PubMed/Medline, Scopus, and Google Scholar from inception until April 2020. The combination of MESH (Medical Subject Headings) and non-MESH terms were applied as follows: ("Zinc ") AND ("Antioxidants" OR "Reactive Oxygen Species" OR "Reactive Nitrogen Species" OR "Oxidative Stress" OR "Nitric Oxide" OR "Malondialdehyde" OR "Superoxide Dismutase" OR "Glutathione" OR "Glutathione Peroxidase" OR "Glutathione Reductase" OR "Oxidative Stresses" OR "stress oxidative" OR "GR" OR "Glutathione Lipoperoxidase" OR "GPx" OR "GSH-Px" OR "Glutathione" OR "SOD" OR "Malonyldialdehyde" OR "Malonylaldehyde" OR MDA OR

"Nitrogen Monoxide" OR "Mononitrogen Monoxide" OR "NO" OR "Reactive Nitrogen Species" OR "Active Oxygen") AND ("Random Allocation" OR "Single-Blind Method" OR "Double-Blind Method" OR "Cross-Over Studies" OR "Clinical Trials as Topic" OR RCT OR "Intervention Studies" OR "intervention" OR "controlled trial" OR "randomized" OR "randomized" OR "random" OR "randomly" OR "placebo" OR "assignment" OR "Cross-Over"). No date and language restriction was applied. In addition, we hand-searched all reference lists of related papers in order to find additional pertinent studies. Activation of the PubMed's e-mail alert service was done as a tool to find any new articles that may have looked on this after our initial search.

Selection criteria

We included studies that fulfilled the following criteria: 1) randomized controlled trials (RCTs) with either parallel or crossover design; 2) were performed on adult subjects (≥18 years); 3) assessed the effects of oral zinc supplementation on aforementioned oxidative stress indices compared to the control group; 4) reported adequate information on pre- and post-supplementation for OS biomarkers in both intervention and control groups.

Articles were excluded if 1) were conducted on children, pregnant women or animals; 2) had a non-RCT design such as observational studies, in-vitro studies, letters, conference papers, dissertations, patents, and protocol studies; 3) were not have any control group to compare the results with intervention group; 4) contained incomplete information on the selected outcomes in zinc or control groups; 5) or surveyed the effects of zinc drugs, herbs, or supplements (where the pure effect of zinc intervention could not be discovered).

Data extraction

Two independent authors (SMM and MRA) obtained the following information from all qualified studies and data were moved into a standardized spreadsheet: first author's name, year of

publication, length of the trial, mean age and gender of participants, study location, design of the study (parallel or crossover), participant's health condition, details of the intervention including dosage and type of zinc supplemented, number of cases and controls, and Mean ± standard deviation (SD) and/or changes of the outcome including MDA, TAC, GSH, and NO in the intervention and control groups at baseline, post-intervention. If any studies provided inadequate data for meta-analysis, we sent emails to the authors at least three times, one month apart, and if we did not receive a response, we excluded the article from the analysis. Disputes were resolved through discussion between the review authors or resolved by a chief reviewer (AE). If an article did not have sufficient data for the analysis, we sent emails to the corresponding author, and if we did not receive a response, so we excluded the article. Trials with multiple dosages of intervention were included as a separate article in the meta-analysis. Moreover, trials that provided effects for varying periods, the longest duration of intervention was considered.

Risk of bias

Two independent reviewers (SMM and MRA) evaluated the risk of bias using the Cochrane Collaboration Risk of Bias guideline [24]. The methodological features considered were: random sequence generation, concealed allocation, blinding of participants, investigator and outcome assessment, incomplete outcome data, selective reporting, and other biases. These items were classified as low risk of bias, high risk of bias or unclear. Studies with a low-risk of bias for all domains were regarded as good quality; studies with one high-risk criterion or two unclear criteria were regarded as fair and studies with two or more high-risk or unclear items were regarded as poor quality (**Table 1**).

Statistical analysis

The mean changes and SDs of serum MDA, TAC, GSH and, NO levels were utilized to calculate the overall effect size. The overall effect sizes were assessed using the random-effects model by DerSimonian and Laird [25] and expressed as weighted mean differences (WMDs) and 95% confidence interval (CI). In studies where the within-group mean changes were not reported, we calculated it separately by subtracting the final mean from the baseline mean value in each group. The SDs of the mean difference was also calculated using the following formula: SD change= square root [$(\text{SD baseline})^2 + (\text{SD final})^2 - (2\times 0.5 \times \text{SD baseline} \times \text{SD final})$] [26]. Moreover, we used Get. Data Graph Digitizer version 2.24 to extract numerical estimates from graphs [27].

We evaluated the heterogeneity between study-specific estimates using the Cochrane's Q-test and the I^2 index, with a significance level set at $p < 0.10$. The potential sources of heterogeneity were explored identified based on type of intervention (sulfate, gluconate, elemental), zinc dosage (< 50 mg/d / ≥ 50mg/d), study duration (<3 months / ≥3 months), and Participant's mean age (< 60 years / ≥60 years). The potential effects of the dosage and duration of zinc supplementation were examined using fractional polynomial modeling in the non-linear dose-response analysis [28]. To explore the impact of each study on the pooled effect size, sensitivity analyses by excluding the studies one by one were conducted [29]. Publication bias was assessed using the funnel plot examination as well as the Egger's test. All statistical tests were performed using the Stata software (Version 14.0, Stata Corp, College Station, TX) and P-values <0.05 were considered statistically significant.

RESULTS

Study selection

Based on the initial database searches, 2968 citations were identified. After removing 322 duplicates, 2648 records remained for further evaluation. During the primary screening, which was

based on the review of titles and abstracts, 2590 articles were excluded and 58 articles remained for full-text extraction. During the secondary screening, 48 studies were excluded for the following reasons: studies did not report relevant outcome (n=18), Trials were carried out on children, adolescent, and pregnant women (n=6), trials without the placebo group (n=6), those were administered other components in adjunct to the zinc (n=4), trials were performed on the same population (n=2), trials without sufficient information (n=4), and non-RCT studies (n=8). Finally, 10 RCTs were included in the current systematic review and meta-analysis [19-22, 30-35]. Of them, 8 trials have reported the effect of zinc on serum levels of MDA [19-22, 30-33], 4 on serum levels of TAC [19, 20, 33, 34], 4 on serum levels of NO [19, 30, 33, 35], and 3 on serum levels of GSH [19, 21, 33]. The detailed phases of the selection process are displayed in **Figure 1**.

Study characteristics

The detailed characteristics of the ten included trials were summarized in **Table 2**. Totally, 721 subjects, including 370 cases and 351 controls, were participated in these studies. These studies were published between 2007and 2019 and were conducted in the United States [31], France [30], Poland [35], Italy [21], China [32], Iran [19, 20, 22, 33, 34]. The duration of the interventions varied from 4 and 24 weeks, and the dosage of the elemental zinc supplementation ranged from 11 to 100 mg/d. One trial had a cross-over design [20], and the rest of them had a parallel design. Two trials exclusively included women [33, 34], while the rest of them were conducted among both genders. The mean age of participants varied between 23 and 75 years old. With regard to the type of zinc supplementation, five administered zinc as gluconate [21, 30-32, 34], four administered zinc as sulfate [19, 20, 22, 33], one another study used elemental zinc [35]. Apart from healthy participants [21, 30, 31], the included articles also involved subjects with different health status including major thalassemia [22], hemodialysis Patients [20, 32], women with

Polycystic Ovary Syndrome [33], diabetic foot ulcer [19], hypertensive patients [35], and women with premenstrual syndrome [34].

Effect of zinc supplementation on serum levels of MDA

Eight trials (with 9 treatment arms) including 596 subjects (305 cases and 291 controls) examined the effect of zinc supplementation on serum MDA concentrations. The pooled effect size using random-effects model showed that zinc supplementation significantly reduced serum MDA activities compared to the placebo (WMD: -0.42 μmol/L; 95% CI: -0.71 to -0.13; $P = 0.005$), with a significant degree of heterogeneity between studies (I^2= 90.1%, P<0.001). Although the sources of heterogeneity did not find by several subgroups, the results indicated a greater decrement in serum MDA concentrations in trials administered ≥50 mg/d elemental zinc (WMD: -0.58 μmol/L; 95% CI: -0.71 to -0.45; P<0.001), compared with trials using < 50 mg/d elemental zinc (WMD: -0.17 μmol/L; 95% CI: -0.27 to -0.07; P=0.001). Greater reduction in serum MDA levels was also found in trials that lasted <3 months (WMD: -0.89 μmol/L; 95% CI: -1.25 to -0.54 P<0.001), those that were carried out on individuals <60 years old (WMD: -0.64 μmol/L; 95% CI: -0.77 to -0.50 P<0.001), and trials that prescribed the sulfate form (WMD: -0.58 μmol/L; 95% CI: -0.71 to -0.45 P<0.001). The detailed results for subgroup analyses were summarized in **Table 3**.

Effect of zinc supplementation on serum levels of TAC

The effect of zinc supplementation on serum TAC concentrations was reported in four trials involving a number of 298 subjects (149 cases and 149 controls). The overall meta-analysis based on the random-effects model showed a significant effect of zinc supplementation in increasing serum TAC levels (WMD: 225.96 mmol/L; 95% CI: 68.42 to 383.5; $P = 0.005$). Also, a considerable between-studies degree of heterogeneity was observed (I^2= 92.3%, P<0.001), and was not explained by several subgroup analyses. According to subgroup analyses, there was no

difference between the subclass of trials using ≥50 mg/d elemental zinc dosage (WMD: 258 mmol/L; 95% CI: 209 to 307; P<0.001) and those that using <50 mg/d elemental zinc dosage (WMD: 260 mmol/L; 95% CI: 171 to 348; P<0.001). Moreover, there was no difference between the other subgroups.

Effect of zinc supplementation on serum levels of GSH

The pooled results of three trials (with 4 treatment arms), involving 252 participants, showed GSH levels significantly increased after zinc intervention (WMD: 49.99 μmol/L; 95% CI: 2.25 to 97.73; *P* = 0.04), with a moderate heterogeneity between studies (I^2= 54.5%, P=0.08) (**Figure 2**). The heterogeneity disappeared when subgroup analysis was performed by type of intervention (I^2= 0.0%, P=0.76), intervention dosage (I^2= 0.0%, P=0.76), study duration (I^2= 0.0%, P=0.76), and Participant's mean age (I^2= 0.0%, P=0.76). Based on subgroup analyses, a significant increment in GSH was found in trials using <50 mg/d zinc supplement (WMD: 58.2 μmol/L; 95% CI: 16.7 to 99.6; P = 0.006), in studies lasted ≥3 moths (WMD: 58.2 μmol/L; 95% CI: 16.7 to 99.6; P = 0.006), trials were done on subjects with a mean age of ≥60 years (WMD: 58.2 μmol/L; 95% CI: 16.7 to 99.6; P = 0.006), and trials that administered the gluconate form (WMD: 58.2 μmol/L; 95% CI: 16.7 to 99.6; P = 0.006).

Effect of zinc supplementation on serum levels of NO

There were four trials involving 222 individuals (113 cases and 109 controls) that compared NO activity between zinc administration and control group. The overall estimates showed NO levels did not significantly change after zinc intervention (WMD: -1.66 μmol/L; 95% CI: -5.89 to 2.57; *P* = 0.44), with a high heterogeneity between studies (I^2= 68.1%, P=0.02) (**Figure 2-B**). The potential source of heterogeneity was identified based on subgroup analysis was the intervention dosage (I^2=42.9%, P=0.18). However, NO levels significantly decreased in trials administered less

than 50 mg/d zinc (WMD: -1.90 μmol/L; 95% CI: -3.48 to -0.32; P = 0.02). There was no difference between the other subsets.

Dose-response analysis

Following dose-response evaluation, a significant non-linear relationship between the intervention dosage of zinc and serum MDA levels was found ($P_{non\text{-}linearity}$ = 0.001). However, significant associations were not observed for other outcomes.

Sensitivity analysis and publication bias

To discover the impact of each individual study on the combined effect size, we stepwise excluded each trial from the overall analysis. We observed the combined effect sizes did not change significantly.

Evaluation of publication bias by visual inspection of funnel plot illustrated evidence of a slight asymmetry in the plots for TAC, but not for other parameters. However, the Egger's test suggested no evidence of publication bias for studies examining the effect of zinc supplementation on serum concentrations of MDA (P=0.53), TAC (P=0.15), GSH (P=0.74), and NO (P=0.89).

DISCUSSION

In contemporary work, the clinical effectiveness of zinc supplementation on oxidative stress biomarkers has been shown the somewhat equivocal. Indeed, although there is evidence to suggest beneficial effects may be elicited following supplementation [19, 20], an almost equal number of studies have reported no evident favorable effects [21, 22]. It is conceivable that such equivocality me be residual confounding manifest through varying study designs, dosages, and time of follow-up or the use of diverse measurement tools. To our knowledge, there has been no systematic review and meta-analysis that has analyzed the effect of zinc supplementation on oxidative stress parameters, and thus, to address the equivocality and in an effort to provide consensus, this study

sought to summarize all published randomized controlled trials that evaluated the effects of zinc supplementation on four oxidative stress biomarkers (MDA, TAC, GSH, and NO). Accordingly, we found that MDA, TAC, and GSH were significantly improved following zinc supplementation, whilst NO also significantly improved, but only in dosages <50mg/d.

We found that serum MDA concentrations decreased to a greater extent in trials that administered ≥50 mg/d elemental zinc, whilst larger reductions were evident in trials that lasted <3 months, individuals <60 years old, and when zinc was administered in sulfate form. For GSH, in trials using zinc <50 mg/d, with trial durations ≥3 months, in participants ≥60 years, and when zinc was administered in the gluconate form, greater increases were evident. However, for TAC, our subgroup analyses did not discern the source of heterogeneity. Whilst for NO, dosages of zinc <50mg/d elicited significant improvements. Our results demonstrate that zinc may be efficacious in the improving oxidative stress biomarkers. Indeed, in previous studies, supplementation with zinc (30mg/day) has been reported to reduce oxidative stress, and markedly improve zinc status [REF, REF, REF], whilst specifically in diabetic patients, recorded as having normal baseline zinc levels, high doses of zinc (100mg/day) were reported to be efficacious in increasing zinc levels [REF]. This supports the findings of the present meta-analysis of RCT's, where our subgroup analyses demonstrated that dose was an important factor. Contrastingly, however, no beneficial effect on oxidative stress and antioxidant defenses was found, as part of the Zenith study, in middle-aged and elderly subjects who received zinc supplementation [REF]. It is possible that, because only healthy participants were included in the aforementioned Zenith study, the highest dosage of zinc (30 mg/day) was not sufficient to significantly alter antioxidant status. Notwithstanding, however, as part of the same overarching project, Mariani et al found that, after zinc supplementation, zinc-dependent enzyme activity and plasma zinc concentration were

significantly higher post supplementation. Thus, highlighting the potential beneficial effects of zinc supplementation on, not only zinc levels, but also, zinc-dependent antioxidant enzymes, in healthy elderly subjects. Furthermore, previous systematic reviews and meta-analyses have identified that zinc supplementation may yield additional beneficial effects on serum levels of LDL cholesterol, triglycerides, total cholesterol, insulin resistance, C-reactive protein concentrations, and systolic blood pressure [15-18].

Zinc is an essential micronutrient required in human metabolism, and can facilitate protein folding, catalyze over 300 enzymes, and is necessary for gene expression regulation [36]. Zinc is known to play antioxidant and anti-inflammatory roles in the human body [37, 38], and mechanistically, studies have highlighted the role of zinc in glutathione peroxidase regulation and metallothionein expression, in addition to zinc acting as a co-factor for superoxide dismutase. Moreover, in the cell membrane, zinc competes with copper and iron, inhibits NADPH-oxidase enzymes, and therein reduces inflammation and hyperglycemia [39, 40]. A further mechanism by which zinc is purported to act as an antioxidant is by impacting glutamate-cysteine ligase expression, which is regarded as the rate-limiting enzyme of *de novo* glutathione synthesis. Indeed, this has a two-fold effect, neutralizing free radicals either directly via glutathione, or indirectly acting as a cofactor for glutathione peroxidase [41]. In cultured human cells, 100–150 mM zinc administration has been shown to upregulate the mRNA levels of glutamate-cysteine ligase via a nuclear factor erythroid 2 related factor 2-dependent pathway [42], suggesting that zinc is capable of modulating total cellular glutathione concentration [43]. Further, several publications have demonstrated that zinc status may be changed in different pathophysiological disorders, such as obesity, diabetes, and hypertension [44]. For instance, Habib et al. reported high concentrations of MDA in overweight/obese patients vs. control group [45]. Relatedly, the effect of zinc supplementation in

the improvement of insulin sensitivity is proposed to be influenced by antioxidant status. Indeed, insulin resistance is strongly associated with increased lipid peroxidation and free radical formation [46-48], where increases in free radical concentration and decreases in antioxidants deleteriously impact on oxidative stress, particularly in diabetic patients. Moreover, there is empirical evidence to suggest oxidative stress might conceivably be associated with decreases in the synthesis of zinc-containing antioxidant enzymes, including superoxide dismutase and glutathione peroxidase [49]. Therefore, it appears that zinc supplementation may confer a number of beneficial outcomes, beyond just antioxidant biomarkers, as highlighted in the present study.

Strengths and limitations

The main strength of this study is that it is, to our knowledge, the first to examine the clinical efficacy of zinc supplementation on oxidative stress biomarkers. We performed several subgroup analyses to show the effects of different subgroups and to identify the potential sources of heterogeneity. we also conducted the dose-response analysis to detect an optimal dosage of zinc supplementation. However, despite the strengths evident in our study, there are some possible limitations that must be appreciated. For instance, there was relatively large heterogeneity in study designs, where a wide array of zinc dosages, types, and study durations were employed. We did, however, endeavor to control for these differences. In addition to these outcomes, there are other parameters to measure oxidative stress that was not included in this meta-analysis due to the few numbers of studies. In addition, due to the small number of studies and related sample sizes, the results of the studies might undermine.

Conclusion

This systematic review and meta-analysis highlights the potential efficacy of zinc for the improvement of oxidative stress markers. Indeed, we found that MDA, TAC, and GSH were

significantly improved following zinc supplementation, whilst NO also significantly improved, but only in dosages <50mg/d. However, the strength of effect on oxidative biomarkers changes according to characteristics, such as; trial duration, dosage, age of participants, health condition of participants, and type of zinc. Thus, we recommend that, whilst health care providers and clinicians may be able incorporate zinc into treatment or management regimens, individualized information must be considered prior to any prescription or advocation.

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