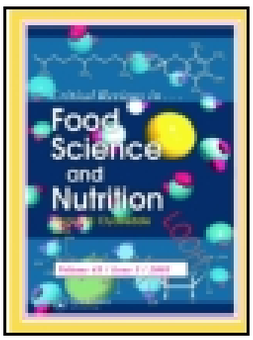

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Alireza Jafari, Zeinab Noormohammadi, Mohammadreza Askari & Elnaz Daneshzad (2020): Zinc supplementation and immune factors in adults: a systematic review and meta-analysis of randomized clinical trials, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2020.1862048

To link to this article: https://doi.org/10.1080/10408398.2020.1862048

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2020.1862048

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REVIEW

Zinc supplementation and immune factors in adults: a systematic review and meta-analysis of randomized clinical trials

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ABSTRACT

Purpose: This systematic review and meta-analysis aimed to investigate the effect of zinc supplementation on immune factors in randomized controlled trials.

Methods: A comprehensive search was done in PubMed, Scopus, Web of Science, Embase, and Cochrane databases up to December 2020. We used standard and weighted mean differences and 95% confidence intervals for net changes in selected parameters of immune responses. Subgroup analysis was used to find heterogeneity.

Result: Overall, 35 RCTs comprising 1995 participants were eligible for this meta-analysis. There was a significant reduction of circulating CRP (WMD: −32.4; 95% CI: −44.45 to −19.62, $p < 0.001$), hs-CRP (WMD: −0.95; 95% CI: −1.01 to −0.89, $p < 0.001$), Neutrophil levels (SMD: −0.46; 95% CI: −0.90 to −0.01, $p = 0.043$), following zinc supplementation. CD4 level also increased significantly, (WMD: 1.79; 95% CI: 0.57 to 3, $p = 0.004$). Zinc supplementation had no significant effect on WBC (SMD: −0.66; 95% CI: −1.67 to 0.36, $p = 0.204$), lymphocyte (WMD: 1.86; 95% CI: −0.86 to 4.58, $p = 0.181$), monocyte levels (SMD: −0.16; 95% CI: −0.07 to 0.39, $p = 0.167$), CD3 (SMD: 0.37; 95% CI: −0.49 to 1.22, $p = 0.399$).

Conclusion: Zinc supplementation decreased the CRP, hs-CRP and TNF-α, IL-6, neutrophil and increased CD3 and CD4 level significantly.

KEYWORDS
Zinc; immune system; adult; systematic review; meta-analysis

Introduction

Zinc is an essential trace element that plays a cruicial role in various biological processes such as cell signaling, cell proliferation, cell activation, gene expression, protein synthesis, and apoptosis. It is also essential for normal development and regulating the immune cells (Prasad 2000; Bogden 2004; Haase, Mocchegiani, and Rink 2006; Mocchegiani et al. 2009). The first study to assay intracellular signaling regulation by zinc and zinc-induced changes in immune cell function was done in the late 1970s (Brewer et al. 1979; Wellinghausen and Rink 1998). Strong evidence presents an association between marginal-to-moderate zinc deficiency and several infectious diseases such as pneumonia, infectious diarrhea (Overbeck, Rink, and Haase 2008; HöNSCHEID, Rink, and Haase 2009; Bhandari et al. 1996; Ruel et al. 1997; Fraker et al. 2000), the incidence of allergies (Rink 2011; Richter et al. 2003), autoimmune diseases, and impairs immunity (Overbeck, Rink, and Haase 2008; Gammoh and Rink 2017; Sandström et al. 1994; Foster and Samman 2012; Fraker et al. 1986; Schlesinger et al. 1992; Castillo-Duran et al. 1987). Animals and human studies identified an altered zinc status can affect immunocompetence (Mocchegiani et al. 2008; Haase and Rink 2009a).

Numerous studies described the role of zinc in both innate and adaptive immune responses. It is essential, especially for proper T and B cell development (Osati-Ashtiani, King, and Fraker 1998; King et al. 2005). Many transcription factors are zinc finger proteins and involved in innate and adaptive immune cell functions; this suggests an additional direct or indirect role of zinc in immunity (Maywald et al. 2017; Zhang et al. 2015). In vitro study showed that zinc improves CD34+ cell progenitors' development toward natural killer (NK) cells (Muzzioli et al. 2009).

Recently several studies suggest using zinc supplementation in the prevention and or treatment of diseases related to immune system dysfunction (Haase and Rink 2009a; Prasad 2014a). Additionally, in elderly individuals, zinc supplementation has been used successfully to restore immune function (Duchateau et al. 1981; Boukaïba et al. 1993). A study by Sazawa et al. showed that oral zinc supplementation for 120 days improved two classic parameters of cell-mediated immune competence; the number of circulating T-lymphocyte, especially CD4 (Sazawal et al. 1997). Newly, zinc administration in physiological doses lowers transplant

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Supplemental data for this article can be accessed at https://doi.org/10.1080/10408398.2020.1862048.

rejections is the capacity of zinc to induce and stabilize a specific subpopulation of Th cells, namely regulatory T cells (Treg) in vitro and in vivo (Rosenkranz et al. 2016b; Maywald et al. 2017; Rosenkranz et al. 2016a; Maywald, Wang, and Rink 2018; Faber et al. 2004; Rosenkranz et al. 2017).

On the other hand, Zinc supplementation could not restore the immune parameters in a study conducted in hemodialysis patients (Turk et al. 1998a). Zinc supplementation showed no increase in absolute numbers of circulating T lymphocytes, T lymphocyte subpopulations during head and neck radiation therapy (Sangthawan, Phungrassami, and Sinkitjarurnchai 2015). Moreover, another study in healthy adult man, zinc does not affect circulating levels of peripheral blood leucocytes and lymphocyte subsets (Bonham et al. 2003). The impact of zinc on inflammatory processes was also controversial among the studies (Ranjbar et al., 2014; Freiberg et al. 2020).

However, zinc deficiency-related immunological outcomes are well known, and investigation about zinc supplementation and immune function is arising; currently, no up-to-date systemic reviews or meta-analyses are examining the effect of zinc supplementation on the immune system, and the results are controversial; therefore, the present study aimed to systematically assess the effects of zinc supplementation on immune factors such as lymphocytes, monocytes, etc.

Methods

The present meta-analysis was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Moher et al. 2010).

Search strategy

We systematically searched studies published prior to December 2020 in Medline/PubMed, ISI Web of Knowledge, Scopus, and Cochrane. We used the following keywords: ["zinc" OR "zinc ione" OR "zinc supplementation" OR "Zn" OR "Zn supplementation"] AND ["immune system" OR "immunity" OR "immune response" OR "immune function" OR "immune cells" OR "neutrophils" OR "monocytes" OR "lymphocytes" OR "CD4-CD8 Ratio" OR "CD4-Positive T-Lymphocytes" OR "CD8-Positive T-Lymphocytes" OR "Tumor Necrosis Factors" OR "C-Reactive Protein" OR "c reactive protein" OR "CRP" OR "high-sensitivity C-reactive protein" OR "hs-CRP" OR "Interleukin-6" OR "il-6" OR "white blood cells"]. The reference lists of related reviews and articles were also searched manually for additional eligible studies. The search was not restricted to publication date or language.

Eligibility and study selection

Two investigators independently reviewed the titles and abstracts of all identified articles and judged the eligibility of articles. The authors included randomized clinical trials (RCTs) which were; 1) reported one of the immune function markers at least; 2) included adult participants (>18 y) 3) mentioned the dosage of zinc supplementation 4) published in English, and 5) reported means, median, standard deviation (SD), standard error (SE), or interquartile range (IQR) of immune function markers for the zinc supplemented and control groups at baseline and the end of the study or mean changes.

We excluded conference papers, letters, review articles, observational studies, duplicate publications, animal or cell cultural studies, and unrelated studies.

Data extraction and quality assessment

Two independent investigators reviewed and extracted the following information from eligible studies: First author's name, publication year, type of the RCTs, duration of intervention, country, participant age-range and(or) mean age (years), participant gender, different forms of zinc supplementation, number of participants in both intervention and control groups, mean and SD of the immune factors, and the dose of zinc supplementation.

To assess the quality of the studies included, we used the Risk of Bias *2* (*RoB 2*) risk of bias tool (Sterne et al. 2019) based on randomization process, deviation from the intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Two independent investigators reviewed the included articles. Any discrepancies were resolved through discussion under the supervision of a senior author.

Statistical analysis

Mean difference (MD) was used as the main measure to summarize zinc supplementation effect on the outcomes. For studies that did not report SD, it was estimated by SE $\times \sqrt{n}$ (n = number of participants). The fixed effects and random-effects models were used To calculate the pooled estimate of MD with a 95% confidence interval (CI). We also used SMD due to variation in the unit of some factors. I^2 test was used to assess heterogeneity. $I^2 > 50\%$ was assumed to represent heterogeneity among studies (Cumpston et al. 2019). If the heterogeneity of the studies was not significant, the fixed effects estimation was reported. Subgroup analysis and meta-regression were conducted to identify sources of between-study heterogeneity and the influence of several factors on the pooled effect sizes. Subgroup analysis was conducted based on studies design, duration (8 weeks), continent, gender, population, dose (50 mg/d), type, form, and deficiency status. The sensitivity analysis of results was used to explore heterogeneity among trials. Publication bias was assessed by visual inspection of funnel plots and Egger's regression test. If publication bias was suspected, we apply the trim and fill method to adjust the effect size. The non-linear potential effect of zinc dosage and duration were examined using fractional polynomial models. All analysis was performed using STATA software (version 12 StataCorp

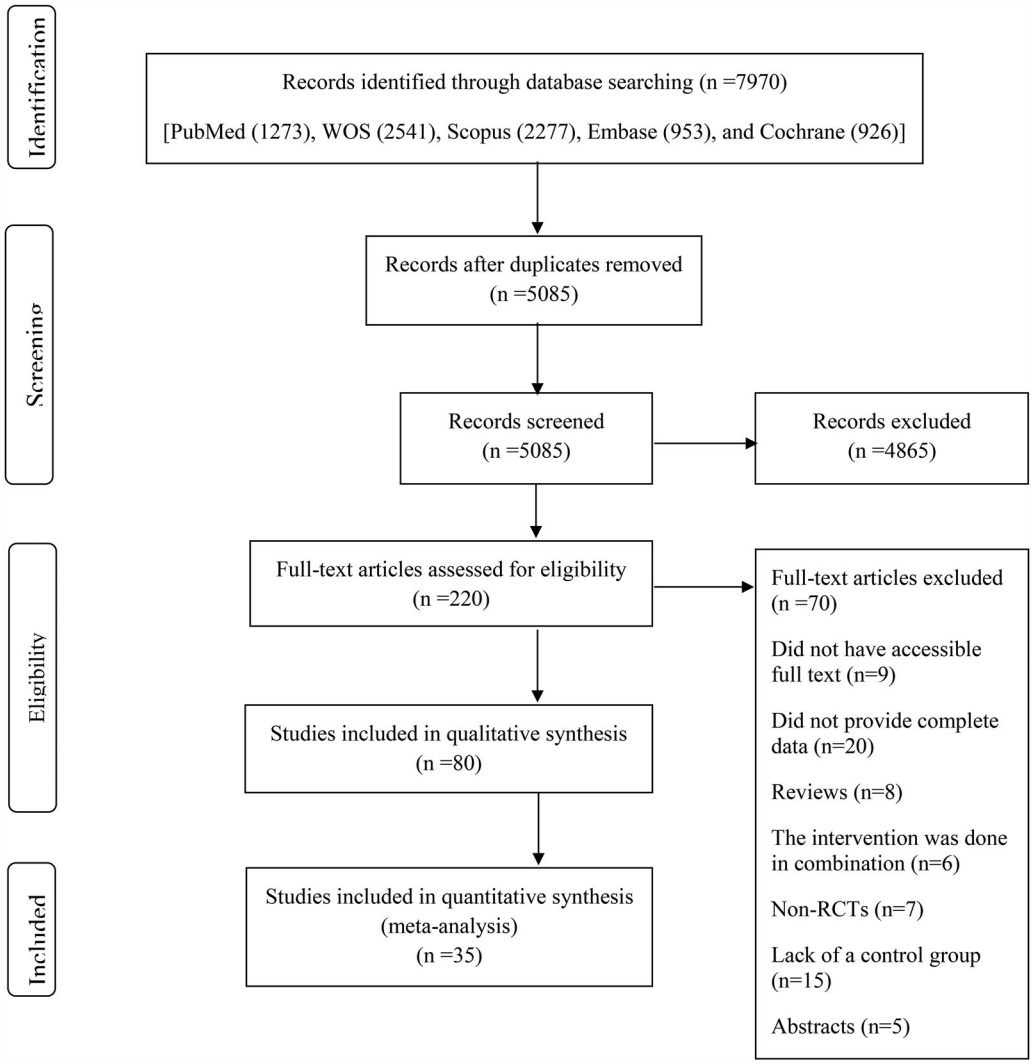

Figure 1. Literature search flow chart.

LP, College Station, TX, USA). P value< 0.05 was considered statistically significant.

Results

Study selection

We identified 7970 articles through our systematic literature search results. After removing duplicates, 5085 articles remained for screening. While screening title and abstract, 4928 publications were excluded. Then, after full-text evaluation of the remaining studies, 122 excluded for the following reasons: did not have accessible full text (n = 9), did not provide complete data (n = 20), reviews (n = 8), the intervention was done in combination (n = 6), non-RCTs (n = 7) lack of a control group (n = 15) and abstracts (n = 5). Among them, 64 included in qualitative and 35 were included in the metanalysis. The flowchart of study selection is shown in Figure 1.

Study characteristics

eneral characteristics of thirty-five included studies are described in Table 1. All trials were in English and had a control group. These articles were published from 1981 (Duchateau et al. 1981) to 2020 (Freiberg et al. 2020). Studies were conducted in Iran (Rashidi et al. 2009; Pourteymour Fard Tabrizi et al. 2011; Ranjbar et al., 2014; Jamilian et al. 2016; Momen-Heravi et al. 2017; Khazdouz et al. 2018; Hadadi et al. 2019; Khorsandi et al. 2019; Jafari, Amani, and Tarrahi 2020), USA (Prasad et al. 2007; Bao et al. 2008), Bangladesh (Islam et al. 2016), Italy (Mocchegiani et al. 1995; Fortes et al. 1998; Provinciali et al. 1998; Iovino et al. 2018), India (Khan et al. 2013), Indonesia (Pakasi et al. 2010), Thailand (Asdamongkol, Phanachet, and Sungkanuparph 2013; Meksawan, Sermsri, and Chanvorachote 2014; Sangthawan, Phungrassami, and Sinkitjarurnchai 2015), Turkey (Turk et al. 1998b), China (Guo and Wang 2013), and other countries. The sample size of studies ranged from 17 (Meksawan, Sermsri, and Chanvorachote 2014; Mei et al. 1991) to 127 (Freiberg et al. 2020). Data were pooled from 64 eligible studies, including participants (1019 cases and 976 control) aged from 18 to 100 (Provinciali et al. 1998) years old. The intervention duration in these studies ranged from 1.5 (Mei et al. 1991) to 72 (Freiberg et al. 2020) weeks. 8 (Bao et al; Bonham et al. 2003; Duchateau et al. 1981; Fortes et al. 1998; Hodkinson

et al. 2007; Mujica-Coopman et al. 2015; Prasad et al. 2007; Provinciali et al. 1998) studies conducted in healthy patients, 5 in HIV(Asdamongkol, Phanachet, and Sungkanuparph 2013; Green et al. 2005; Hadadi et al. 2019; Mocchegiani et al. 1995; Freiberg et al. 2020), and 5 in diabetes (Foster, Petocz, and Samman 2013; Islam et al. 2016; Khan et al. 2013; Meksawan, Sermsri, and Chanvorachote 2014; Momen-Heravi et al. 2017) subjects. One study was conducted exclusively in men (Bonham et al. 2003) and 12 in women. Zinc was administered as sulfate (Bobat et al. 2005; Cvijanovich et al. 2016; Duchateau et al. 1981; Fortes et al. 1998; Foster, Petocz, and Samman 2013; Green et al. 2005; Iovino et al. 2018; Islam et al. 2016; Jamilian et al. 2016; Khan et al. 2013; Khazdouz et al. 2018; Khorsandi et al. 2019; Meksawan, Sermsri, and Chanvorachote 2014; Mocchegiani et al. 1995; Mujica-Coopman et al. 2015; Pakasi et al. 2010; Pourteymour Fard Tabrizi et al. 2011; Provinciali et al. 1998; Ranjbar et al., 2014; Rashidi et al. 2009; Sangthawan, Phungrassami, and Sinkitjarurnchai 2015; Turk et al. 1998b) gluconate (Bao et al. 2008; Freiberg et al. 2020; Guo and Wang 2013; Hadadi et al. 2019; Hodkinson et al. 2007; Jafari, Amani, and Tarrahi 2020; Kim and Ahn 2014; Momen-Heravi et al. 2017; Prasad et al. 2007; Range et al. 2006), acetate (Bao et al. 2008), and glycine form (Dias et al. 2014), and dose ranged between 5 (Adriani and Wirjatmadi 2014) to 44(Duchateau et al. 1981) mg/d. All studies used a parallel design. Also, 11 (Duchateau et al. 1981; Mei et al. 1991; Mocchegiani et al. 1995; Provinciali et al. 1998; Turk et al. 1998b; Range et al. 2006; Guo and Wang 2013; Asdamongkol, Phanachet, and Sungkanuparph 2013; Khan et al. 2013; Meksawan, Sermsri, and Chanvorachote 2014; Iovino et al. 2018) studies did not perform double-blinding.

Four studies did not find any significant change in IgA (Animashaun et al. 1990; Lazarus et al. 2018; Swanson et al. 1988; Saeedy, Bijeh, and Moazzami 2018), IgG (Animashaun et al. 1990; Holtkamp et al. 1993; Swanson et al. 1988; Saeedy, Bijeh, and Moazzami 2018), and IgM (Animashaun et al. 1990; Swanson et al. 1988; Saeedy, Bijeh, and Moazzami 2018) levels except one. Three studies did not significantly affect IL-1 (Abdulhamid et al. 2008; Foster, Petocz, and Samman 2013; Prasad et al. 2007); however, two (Bao et al. 2008; Guo and Wang 2013) articles indicated a significant reduction. Three (Abdulhamid et al. 2008; Bao et al. 2008; Rahfiludin et al. 2011) studies did not observe a significant effect on IL-2, while two (Acevedo-Murillo et al. 2019; Ahmad and Al-Ahmare 2016) articles have a significant effect. Five studies(Fortes et al. 1998; Turk et al. 1998b; Green et al. 2005; Sangthawan, Phungrassami, and Sinkitjarurnchai 2015; Iovino et al. 2018) did not significantly affect CD8.

Quality assessment

Table 2 describes the risk of bias assessment based on ROB-2. Fourteen studies did not report clear information about deviation (Duchateau et al. 1981; Mei et al. 1991; Provinciali et al. 1998; Turk et al. 1998b; Range et al. 2006; Guo and Wang 2013; Khan et al. 2013; Meksawan, Sermsri, and Chanvorachote 2014; Iovino et al. 2018), and eleven studies did not report enough data about missing data (Duchateau et al. 1981; Mei et al. 1991; Provinciali et al. 1998; Turk et al. 1998b; Bao et al. 2008; Dias et al., 2014; Kim and Ahn 2014; Meksawan, Sermsri, and Chanvorachote 2014). Twelve of the included studies did not provide information about measurement (Duchateau et al. 1981; Mocchegiani et al. 1995; Provinciali et al. 1998; Turk et al. 1998b; Range et al. 2006; Bao et al. 2008; Guo and Wang 2013; Asdamongkol, Phanachet, and Sungkanuparph 2013; Gülsan et al. 2013; Khan et al. 2013; Meksawan, Sermsri, and Chanvorachote 2014; Iovino et al. 2018). Other sources of bias were low.

Meta-analysis

Tables 1 shows the general characteristics of 52 studies included in meta-analysis among 62 studies.

The effect of zinc on serum levels of CD3

Out of five studies that examined the effect of zinc on CD3, four studies, including 116 intervention and 85 controls, were included. There was no significant effect of zinc on CD3 compared to placebo in random-effect model (SMD: 0.37; 95% CI: −0.49 to 1.22, $P = 0.399$) (Figure 2) ($I^2 = 89.9\%$, $P < 0.001$). Visual inspection of the funnel plot and Egger's linear test shows no evidence of publication bias ($P = 0.592$) (Supplemental Figure 1). Subgroup analysis was performed based on studies duration, countries, gender, population, dose, type, and form. A significant effect was seen in studies with more than eight weeks duration, Asian population, both gender, cancer patients, more than 50 mg/d of doses, sulfate type, sirup form, and zinc-deficient patients.

The effect of zinc on serum levels of CD4

The pooled mean difference from random-effect model on eight datasets including 257 intervention and 253 control, for the effect of zinc on CD4 revealed a significant effect (SMD: 1.11; 95% CI: 0.23 to 1.99, $P = 0.013$), ($I^2 = 93.3\%$, $P < 0.001$) (Figure 3). Moreover, in sensitivity analysis, after removing Mocchegiani et al. (Mocchegiani et al. 1995) the significance and heterogeneity was disappeared (SMD: 0.11; 95% CI: −0.08 to 0.29, $P = 0.251$), ($I^2 = 0\%$, $P = 0.77$). The subgroup analysis showed that design, duration, dose, type, and zinc status were the potential source of heterogeneity (Supplemental Table 2). The zinc effect in studies with single-blind design, European countries, AIDS patients, tablet form, and zinc sufficient patients is significant too. Visual inspection of the funnel plot and Egger's test revealed evidence of publication bias ($P = 0.012$). (Supplemental Figure 2). However, after two studies filled using the trim and fill method, our result was not imputed. A significant effect between dose ($P = 0.006$) and duration ($P = 0.005$) was observed in meta-regression (Supplemental Figures 13 and 14).

Table 1. Demographic characteristics of included studies for qualitative synthesis.

Code/author(year)	Study location	Subjects and gender	Age (y)	Design	Intervention (name and composition)	Dose (mg/d)	Duration(wk.)	Notes about subjects	**Outcome (yes/no)**	Adjustments
Duchateau et al. (1981)	Belgium	M:15 F:15 ZN:15 CON:15	75-86 ZN:81 CON:79.6	(3)	Zinc sulfate	440	4	Healthy	Leukocytes (no) Lymphocytes (no)	N
Mei et al. (1990)	China	M:9 F:8 ZN:10 CON:7	33-66 ZN:48.7 CON:56	(2)	Zinc tablet	20	1.5	Cancer	WBC (no)	N
Mocchegiani et al. (1995)	Italy	M:35 F:22 ZN:47 CON:44	35-77 ZN:57.4 CON:57.1	(3)	Zinc sulfate	200	4	AIDS	$CD4^+$ (yes)	N
Fortes et al. (1998)	Italy	M:13 F:45 ZN:30 CN:28	72-88 ZN:79.5 CON:80.6	(1)	Zinc sulfate capsule	25	12	Healthy	Leukocytes (no) Lymphocytes(no) $CD3^+$ (no) $CD4^+$ (no) $CD8^+$ (no) CD4/CD8 (no) T-lymphocytes (no)	Baseline, BMI, depression, chronic disease, smoking, acute respiratory disease
Provinciali et al. (1998)	Italy	ZN:32 CON:31	64-100 ZN:59.7 CON:54.1	(3)	Zinc sulfate	400	8	Healthy	Leukocytes (no) Lymphocytes (no) Neutrophils (no)	N
Turk et al. (1998)	Turkey	M:10 F:16 ZN:13 CON:13	21-67 ZN:37 CON:46	(2)	Zinc sulfate	120	4	Hemodialysis	$CD3^+$ (no) $CD4^+$ (no) $CD8^+$ (no) CD4/CD8 (no)	N
Bonham et al. (2003)	UK	M:38 ZN:19 CON:19	33-37 ZN:35.8 CON:35.3	(1)	Zinc glycine	30	14	Healthy	$CD3^+$ (no)	N
Green et al. (2005)	Singapore	M:61 F:5 ZN:31 CON:34	31-48 ZN:40 CON:40	(1)	Zinc sulfate capsule	50	8	HIV	$CD4^+$ (no) $CD8^+$ (no)	Baseline zinc and CD4
Range et al. (2006)	Tanzania	ZN:58 CON:48	22-48 ZN:35.2 CON:35.3	(2)	Zinc gluconate tablet	45	8	Pulmonary tuberculosis	$CD4^+$ (no)	Sex, age, HIV status, heavy cultere intensity, alcohol, smoking
Hodkinson et al. (2007)	Ireland	ZN:31 ZN:28 CON:34	55-70 ZN:62.4 CON:62.6	(1)	Zinc gluconate capsule	15 30	24	Healthy	Neutrophils (no) Lymphocytes (no) Monocytes (yes) $CD3^+$ (no)	Sex, age, baseline
Prasad et al. (2007)	USA	M:16 F:33 ZN:24 CON:25	56-74 ZN:65 CON:67	(1)	Zinc gluconate capsule	45	24	Healthy	TNF-α (yes)	N
Bao et al. (2008)	USA	M:22 F:14 ZN:18 CON:18	29-65 ZN:32.6 CON:33.6	(1)	Zinc acetate capsule	75	12	sickle cell	WBC (yes) Lymphocytes (no) Monocytes (no)	N

(*continued*)

Table 1. Continued.

Code/author(year)	Study location	Subjects and gender	Age (y)	Design	Intervention (name and composition)	Dose (mg/d)	Duration(wk.)	Notes about subjects	**Outcome (yes/no)**	Adjustments
Rashidi et al. (2009)	Iran	M:32 F:23 ZN:28 CON:27	ZN:56.4 CON:59	(1)	Zinc sulfate capsule	220	6	Hemodialysis	CRP (no)	N
Bao et al. (2010)	USA	ZN:20 CON:20	58-74 ZN:65 CON:67	(1)	Zinc gluconate capsule	45	24	Healthy	hs-CRP (yes) IL-6 (yes)	N
Pakasi et al. (2010)	Indonesia	ZN:63 CON:77	15-55 ZN:30.9 CON:31.4	(1)	Zinc sulfate capsule	15	12	Severely malnourished pulmonary tuberculosis	CRP (yes) Leucocytes (yes)	N
Pourteymour et al. (2011)	Iran	ZN:30 CON:30	20-45 ZN:27.2 CON:26.9	(1)	Zinc sulfate capsule	50	8	PCOS	hs-CRP (yes) IL-6 (yes)	N
Guo et al. (2012)	China	M:36 F:29 ZN:40 CON:25	49-69 ZN:59 CON:61	(3)	Zinc gluconate capsule	11	8	Hemodialysis	hs-CRP (no) TNF-α (no)	N
Asdamongkol et al. (2013)	Thailand	ZN:13 CON:17	34-56 ZN:47 CON:43	(2)	Zinc pill	15	24	HIV	$CD4^+$ (yes)	N
Foster et al. (2013)	Australia	ZN:12 CON:10	57-73 ZN:65 CON:66	(1)	Zinc sulfate capsule	40	12	Type 2 diabetes mellitus	IL-6 (no) TNF-α (no) CRP (no)	Age, BMI, dietary zink intake
Khan et al. (2013)	India	ZN:23 CON:21	40-69 ZN:56 CON:56.3	(3)	Zinc sulfate capsule	50	8	Type 2 diabetic nephropathy	hs-CRP (yes)	N
Ranjbar et al. (2014)	Iran	ZN:20 CON:17	18-55 ZN:37 CON:37.5	(1)	Zinc sulfate capsule	25	12	Depressed	IL-6 (yes) TNF-α (yes)	N
Dias et al. (2014)	Brazil	M:33 F:21 ZN:27 CON:27	41-82 ZN:62 CON:62	(1)	Zinc glycine pill	30	16	Atherosclerosis	hs-CRP (yes)	N
Kim et al. (2014)	South Korea	ZN:20 CON:20	18-23	(1)	Zinc gluconate tablet	30	8	Obese	hs-CRP (yes) TNF-α (no) IL-6 (yes)	N
Meksawan et al. (2014)	Thailand	ZN:8 CON:9	47-69 ZN:58.7 CON:57.6	(2)	Zinc sulfate capsule	30	8	Type 2 diabetes mellitus	Lymphocytes (no) Monocytes (no) TNF-α (no)	N
Jamilian et al. (2016)	Iran	ZN:24 CON:24	18-40	(1)	Zinc sulfate tablet	50	8	PCOS	hs-CRP (no)	Baseline, age, BMI
Mujica-Coopman et al. (2015)	Chile	ZN:26 CON:28	18-45 ZN:34.9 CON:33.5	(1)	Zinc sulfate capsule	30	12	Healthy	CRP (no)	N
Sangthawan et al. (2015)	Thailand	ZN:35 CON:35	29-78 ZN:62 CON:60	(1)	Zinc sulfate sirup	150	4	Head and neck cancer	WBC (no) Neutrophil (no) Lymphocyte (no) $CD3^+$ (no) $CD4^+$ (no)	N

									$CD8^+$ (no) CD4/CD8 (no)	
Islam et al. (2016)	Bangladesh	M:28 F:27 ZN:28 CON:27	34-52 ZN:42.1 CON:45.5	(1)	Zinc sulfate tablet	30	24	Prediabetes	CRP (yes)	N
Khazdouz et al. (2018)	Iran	M:42 F:18 ZN:46 CON:48	18-65 ZN:32 CON:37	(1)	Zinc sulfate solution	120	2	Severe head trauma	CRP (yes) WBC (yes)	N
Momen-Heravi et al. (2017)	Iran	ZN:30 CON:30	40-85 ZN:58.3 CON:60	(1)	Zinc gluconate capsule	50	12	Diabetic foot ulcer	hs-CRP (yes)	Baseline, age, BMI, ulcer depth
Iovino et al. (2018)	Italy	M:12 F:6 ZN:9 CON:9	47-72 ZN:63 CON:58	(3)	Zinc sulfate tablet	150	12	Hematopoietic stem cell transplant	$CD4^+$ (no) $CD8^+$ (yes)	N
Hadadi et al. (2019)	Iran	M:49 F:44 ZN:49 CON:44	18-60 ZN:38.1 CON:38.6	(1)	Zinc gluconate capsule	50	24	HIV/AIDS	$CD4^+$ (no) CD4/CD8 (no)	Baseline, age, calorie
Jafari et al. (2020)	Iran	ZN:30 CON:30	18-30 ZN:23 CON:22.5	(1)	Zinc gluconate tablet	30	12	Premenstrual syndrome	hs-CRP (no)	Baseline
Khorsandi et al. (2019)	Iran	M:14 F:26 ZN:18 CON:22	18-45 ZN:35.6 CON:32.9	(1)	Zinc sulfate capsule	30	8	Obese	hs-CRP (no) TNF-α (no)	Age, calore, dietary zinc intake
Freiberg et al. (2020)	Russia	ZN:64 CON:63	18-70 ZN:34 CON:34	(1)	Zinc gluconate capsule	14	72	HIV + alcohol	$CD4^+$ (no) IL-6 (no)	Sex, alcohol

1: randomized, double-blinded, placebo-controlled clinical trial; 2: a randomized placebo-controlled clinical trial; 3: randomized clinical trial; M: male; F: female; ZN: zinc group; CON: control group; TNF-α: tumor necrosis factor alfa; CRP: C-Reactive Protein; hs-CRP: high sensitivity C-Reactive Protein; CD: a cluster of differentiation; IL: interleukin; WBC: white blood cell; HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome; PCOS: Polycystic ovary syndrome.

Table 2. Cochrane Risk of bias assessment of randomized controlled trials of the effect of Zinc.

Publication	Randomization process	Deviation from the intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Duchateau et al. (1981)	L	S	S	S	L	S
Mei et al. (1990)	L	S	S	L	L	S
Mocchegiani et al. (1995)	L	L	L	S	L	L
Fortes et al. (1998)	L	L	L	L	L	L
Provinciali et al. (1998)	L	S	S	S	L	S
Turk et al. (1998)	L	S	S	S	L	S
Bonham et al. (2003)	L	L	L	L	L	L
Green et al. (2005)	L	L	L	L	L	L
Range et al. (2006)	L	S	L	S	L	S
Hodkinson et al. (2007)	L	L	L	L	L	L
Prasad et al. (2007)	L	L	L	L	L	L
Bao et al. (2008)	L	L	S	L	L	L
Rashidi et al. (2009)	L	L	S	L	L	L
Bao et al. (2010)	L	L	S	S	L	S
Pakasi et al. (2010)	L	L	L	L	L	L
Pourteymour et al. (2011)	L	L	L	L	L	L
Guo et al. (2012)	L	S	L	S	L	S
Asdamongkol et al. (2013)	L	L	L	S	L	L
Foster et al. (2013)	L	L	L	L	L	L
Khan et al. (2013)	L	S	L	S	L	S
Ranjbar et al. (2014)	L	L	L	L	L	L
Dias et al. (2014)	L	L	S	L	L	L
Kim et al. (2014)	L	L	S	L	L	L
Meksawan et al. (2014)	L	S	S	S	L	S
Jamilian et al. (2016)	L	L	L	L	L	L
Mujica-Coopman et al. (2015)	L	L	L	L	L	L
Sangthawan et al. (2015)	L	L	L	L	L	L
Islam et al. (2016)	L	L	L	L	L	L
Khazdouz et al. (2017)	L	L	L	L	L	L
Momen-Heravi et al. (2017)	L	L	L	L	L	L
Iovino et al. (2018)	L	S	L	S	L	S
Hadadi et al. (2019)	L	L	L	L	L	L
Jafari et al. (2020)	L	L	L	L	L	L
Khorsandi et al. (2019)	L	L	L	L	L	L
Freiberg et al. (2020)	L	L	L	L	L	L

L: low risk of bias; S: some concerns.

The overall effect of zinc in random-effect model on CD4 percent among 134 intervention and 113 control, was not significant (WMD: 1.66; 95% CI: −3.02 to 6.34, P = 0.487) (I^2= 90.6%, P < 0.001) (Figure 4). The subgroup analysis showed that zinc status was the potential source of heterogeneity (Supplemental Table 3) moreover, showed that studies with non-blind design, less than 8 weeks duration, Asian population, hemodialysis patients, less than 50 mg dose, gluconate type, capsule form, and zinc-deficient patients was significant. Visual inspection of the funnel plot and Egger's test revealed no evidence of publication bias (P = 0.844) (Supplemental Figure 3).

The effect of zinc on serum levels of CD4/CD8

Out of six studies that examined the effect of zinc on CD4/CD8, a forest plot of four studies including 137 intervention and 117 control, shows that a non-significant difference in CD4/CD8 ratio with zinc in random-effect model (WMD: 0.12; 95% CI: −0.05 to 0.28, P = 0.163) (I^2= 80.4%, P = 0.002). (Figure 5) even after eliminating Sangthawan et al. had no significant effect (WMD: 0.12; 95% CI: −0.05 to 0.29, P = 0.174) (Sangthawan, Phungrassami, and Sinkitjarurnchai 2015). The subgroup analysis showed that design and type were potential sources of heterogeneity (Supplemental Table 4). Subgroup analysis revealed that, zinc effect in studies with a non-blind design, lower than eight weeks duration, zinc-deficient, and hemodialysis patient is significant. No publication bias was seen from the funnel plot and Egger's test (P = 0.488) (Supplemental Figure 4).

The effect of zinc on serum levels of WBC

Out of six studies that examined the effect of zinc on WBC by random-effect model, the analysis of four RCTs with including 109 intervention and 108 control, revealed that zinc supplementation had no significant effect on WBC (SMD: −0.70; 95% CI: −2.1 to 0.7, P = 0.204) (Figure 6) even after eliminating Khazdouz et al. did not have a significant effect on WBC (SMD: −0.22; 95% CI: −69 to 0.25, p = 0.363) (Khazdouz et al. 2018). The between-study heterogeneity was high (I^2= 95%, P < 0.001). The subgroup analysis showed no potential source of heterogeneity (Supplemental Table 5). However, the result is significant in studies with blind design, cancer patients, less than 50 mg

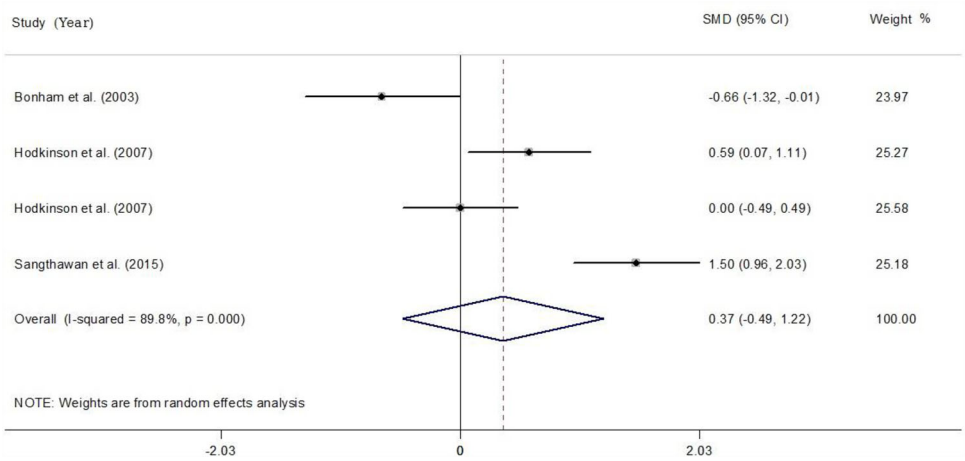

Figure 2. Forest plot of the effect of zinc on CD + 3 using a random-effects model.

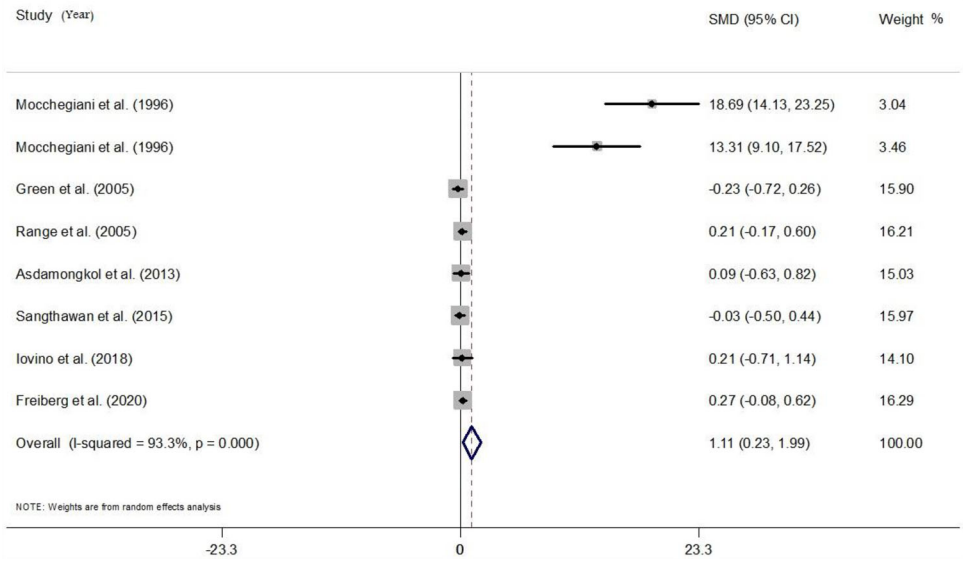

Figure 3. Forest plot of the effect of zinc on CD 4 using a random-effects model.

dose, sirup, tablet form, and zinc deficient. Visual inspection of the funnel plot and Egger's test shows no evidence of publication bias (P = 0.835) (Supplemental Figure 5).

The effect of zinc on serum levels of lymphocyte

The pooled mean difference of random-effect model on seven datasets including 189 intervention and 161 control, for the effect of zinc on lymphocytes revealed a non-significant effect (SMD: 0.23; 95% CI: −0.09 to 0.55, P = 0.0787), without between-study heterogeneity (I^2= 57.1%, P = 0.03) (Figure 7). Visual inspection of the funnel plot and Egger's test did not suggeste a publication bias (P = 0.687) (Supplemental Figure 6). The meta-regression failed to show non-linear dose (P = 0.605) and duration (P = 0.761) (Supplemental Figures 15 and 16).

The effect of zinc on serum levels of monocyte

Out of four studies that examined the effect of zinc on monocytes, three studies including, 77 intervention and 52 control, providing a total of participants, reported monocyte as an outcome measure. Compared with placebo, administering zinc as the intervention was not associated with a significant increase in monocyte levels (SMD: 0.06; 95% CI: −0.25 to 0.37, P = 0.722). We found no heterogeneity among studies (I^2= 0%, P = 0.387) (Figure 8). Visual inspection of the funnel plot and Egger's test shows no evidence of publication bias (P = 0.788) (Supplemental Figure 7).

The effect of zinc on serum levels of neutrophil

Pooled results from four studies including 126 intervention and 101 control, using the random-effect model indicated that zinc as intervention resulted in significant reduction in Neutrophil levels (SMD: −0.46; 95% CI: −0.90 to −0.01, P = 0.043) (I^2= 88.4%, P = 0.023) (Figure 9). The subgroup analysis showed no potential sources of heterogeneity (Supplemental Table 7). Also, in non-blind studies, lower than eight weeks duration, Asian population, cancer patients, more than 50 milligram dosage, sulfate type, tablet form, and deficient patients zinc effect were not significant.

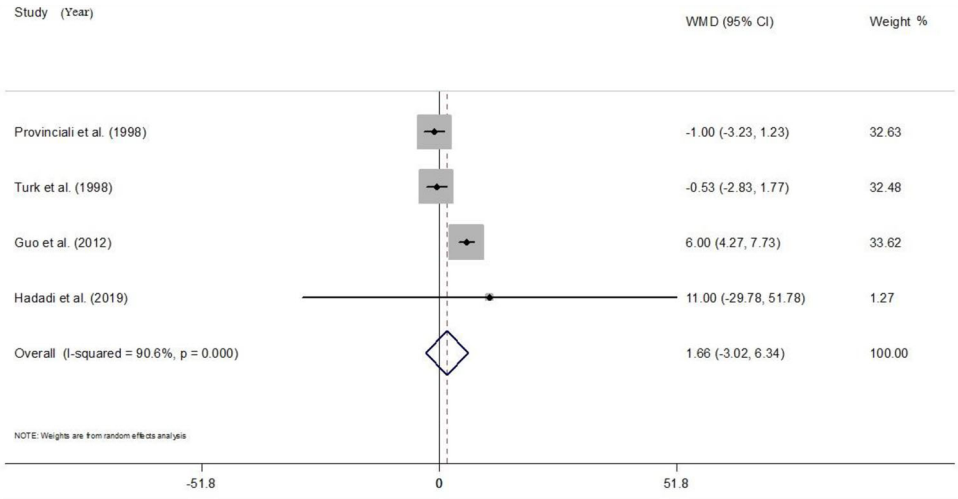

Figure 4. Forest plot of the effect of zinc on CD + 4% using a random-effects model.

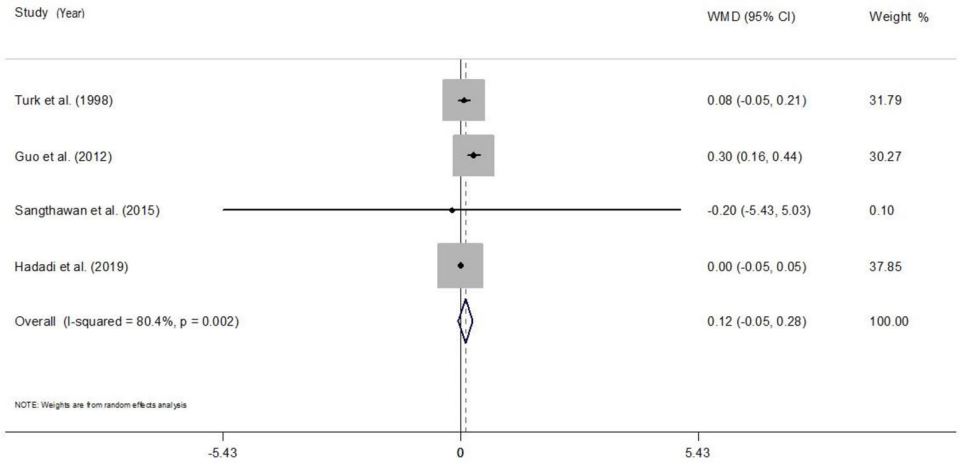

Figure 5. Forest plot of the effect of zinc on CD 4/CD 8 using a random-effects model.

Visual inspection of the funnel plot and Egger's test shows no evidence of publication bias (P = 0.099) (Supplemental Figure 7).

The effect of zinc on serum levels of IL6

Out of 13 studies that examined the effect of zinc on IL6, six with 166 intervention, and 160 control. The overall effect of zinc relative to control on IL6 was −3.15. Heterogeneity between studies was high (I^2= 97.1%, p < 0.001) (Figure 10). No significances were seen in studies with more than eight weeks duration, Australia country, both gender, more than 50 mg dose. Visual inspection of the funnel plot and Egger's test revealed no evidence of publication bias (P = 0.204) (Supplemental Figure 9). Meta-regression for dose (0.325) and duration (0.532) have not a significant non-linear effect (Supplemental Figures 17 and 18).

The effect of zinc on serum levels of CRP

A meta-analysis of six clinical trials including 175 intervention and 191 control show a statistically significant difference in CRP levels between intervention and control groups (WMD: −102.13; 95% CI: −143.65 to −60.60, P < 0.001) (Figure 11) even after eliminating Khazdouz et al. zinc had a significant effect on CRP (WMD: −26.82; 95% CI: −39.26 to −14.37, p = 0.007) (Khazdouz et al. 2018). The between-study heterogeneity was high (I^2= 100%, P < 0.001). No significant effect of zinc supplementation was seen in studies from Australian countries. Visual inspection of the funnel plot and Egger's test shows no evidence of publication bias (P = 0.344) (Supplemental Figure 10). A significant effect for dose (0.009) but not duration (p = 0.263) was observed in meta-regression (Supplemental Figures 19 and 20).

The effect of zinc on serum levels of hs-CRP

Out of 13 RCTs that examined the zincs effect from the random-effect model on CRP, ten trials with 292 intervention and 279 control were included. According to the result of the meta-analysis of ten RCTs, which had reported data on hs0CRP changes, zinc intake resulted in reduced hs-CRP compared with a control group, and the observed effect was significant (WMD: −0.52; 95% CI: −0.57 to −0.47, P < 0.001) (I^2= 99.8%, P < 0.001) (Figure 12) However, we found a non-significant effect of zinc in studies comprising atherosclerosis and premenstrual

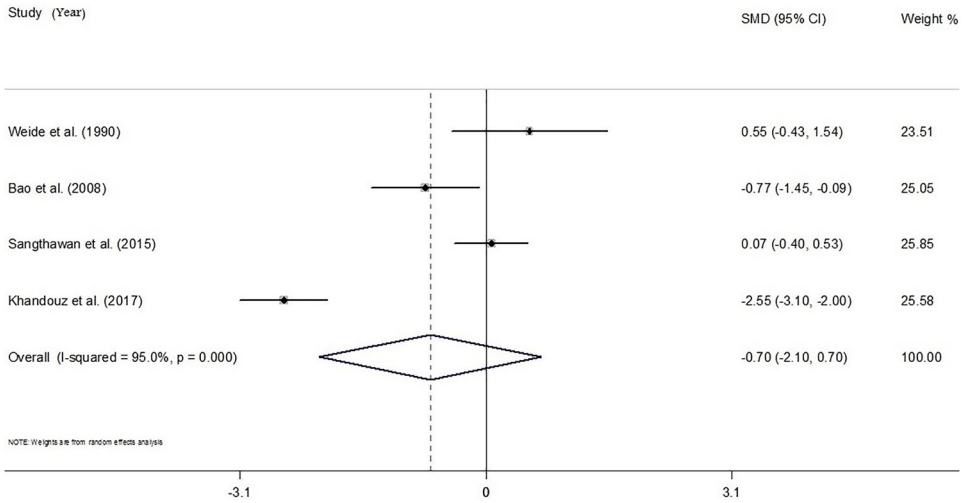

Figure 6. Forest plot of the effect of zinc on WBC using a random-effects model.

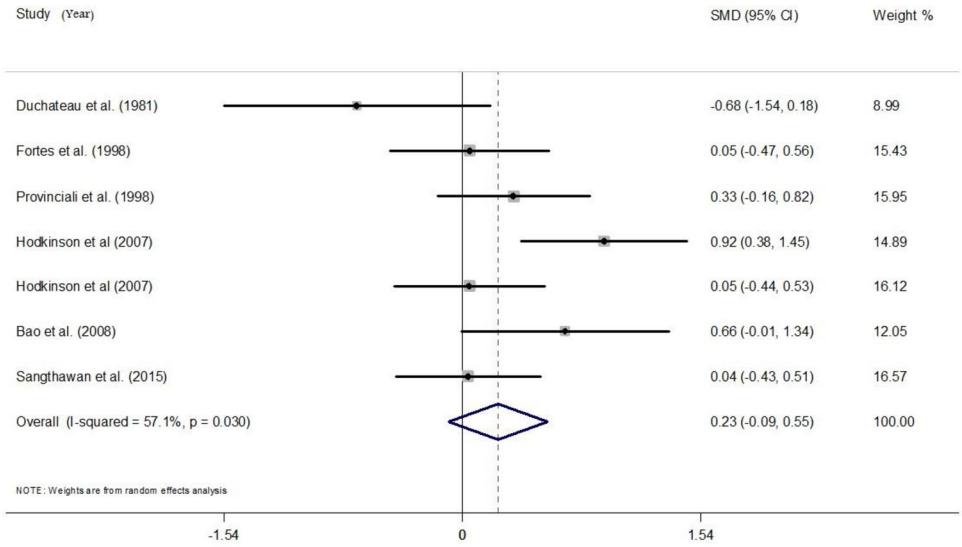

Figure 7. Forest plot of the effect of zinc on Lymphocyte using a random-effects model.

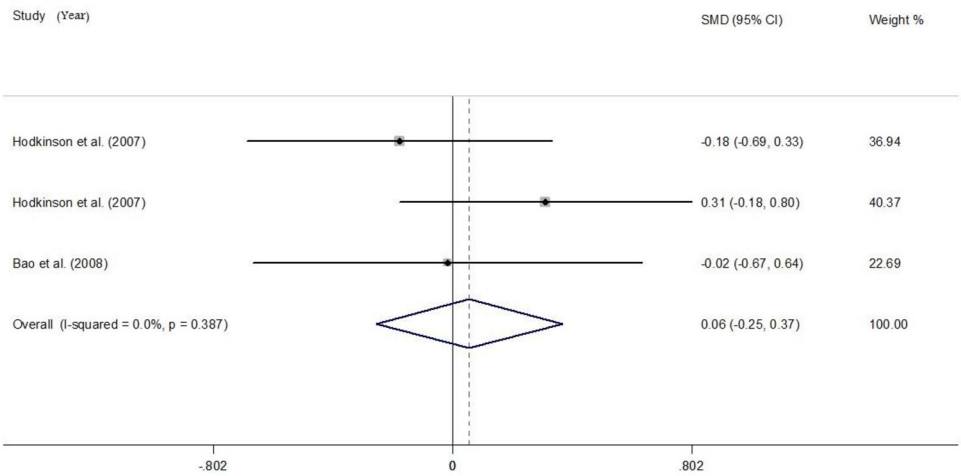

Figure 8. Forest plot of the effect of zinc on Monocyte using a random-effects model.

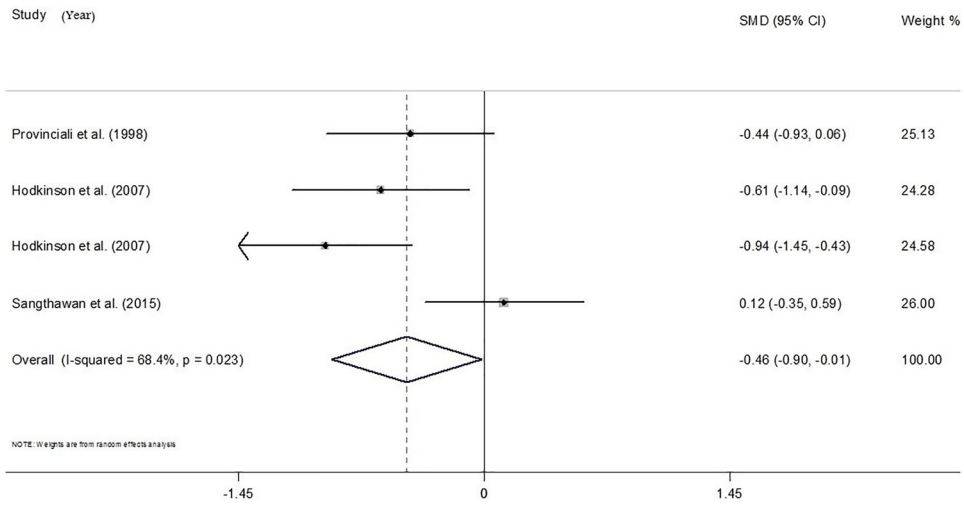

Figure 9. Forest plot of the effect of zinc on Neutrophil using a random-effects model.

syndrome patients, glycine type, and pill form of zinc. Visual inspection of the funnel plot and Egger's test shows no evidence of publication bias (P = 0.110) (Supplemental Figure 11). There was no effect in meta-regression for dose (P = 0.074) and duration (P = 0.43) (Supplemental Figures 21 and 22).

The effect of zinc on serum levels of TNF-α

Out of 13 studies that examined the effect of zinc on TNF, eight studies included 160 intervention and 146 control, in the meta-analysis. The overall effect of zinc on TNF-α from the random-effect model was −8.22 (95% CI: −15.34 to −1.10, P = 0.024) (I^2= 97%, P < 0.001. There was high heterogeneity among studies (I^2= 97.8%, P < 0.001) (Figure 13). In our subgroup analysis, the zinc effect is not significant in non-blind design, more than eight weeks duration, Asian and Australian countries, both gender, CF, and T2DM patients, sulfate, and citrate type, capsule form and zinc sufficient patients. Visual inspection of the funnel plot and Egger's test shows evidence of publication bias (P = 0.037) (Supplemental Figure 12). There was no difference by using trim and fill method. Meta-regression indicated no evidence of dose (P = 0.992) and duration (P = 0.664) response (Supplemental Figures 23 and 24).

Discussion

In the present study, we comprehensively reviewed the effect of zinc administration on different immune system markers, including CD4, CD4/CD8, IL6, CRP, hs-CRP, lymphocyte, monocyte, and TNF-α. Totally 35 randomized controlled clinical trials (RCT) were included in this study. Finally, we found that in zinc supplemented group CD3, and CD4 levels increased, and neutrophil, CRP, and IL6 levels decreased significantly. There were no differences between zinc and placebo groups in other immune factors. Moreover, the analyzed studies indicated that zinc administration significantly increased CD4/CD8 ratio and WBC levels in subjects with zinc deficiency states like hemodialysis and cancer patients.

Our analysis results showed that zinc supplementation could increase CD3 level; however, this finding was non-significant. The subgroup analysis results showed that zinc significantly raised the serum CD3 level in the group that supplemented with the dosage of more than 50 mg/d after more than 8 weeks at least. We observed the same positive effect on cancer and zinc-deficient patients. Our results support the findings of a previous study by Bonomini et al. (Turk et al. 1998), which showed the effectiveness of zinc supplementation on CD3 status, such as earlier reports of Sazawel et al. that also revealed the significant positive outcomes in cellular immunity (Sazawal et al. 1997).

Serum CD4 levels were positively affected by zinc administration. Nevertheless, the overall effect of zinc on CD4 percentage was not significant. Subgroup analysis showed that the CD4 level was raised in the Asian population, hemodialysis, and zinc-deficient patients after zinc supplementation. It could be interpreted that health background highly affects the results. Regarding the effect of zinc supplementation in the CD4 level because there is not a positive impact of zinc in subjects with normal zinc state. Notably, zinc induces regrowth of the thymus to increase thymic hormone production (Haase and Rin). Moreover, the observation of an increase in the number of circulating T-lymphocytes, especially CD4, after zinc supplementation may be explained by the direct effect of zinc on the lymphocyte membrane, which affects the maturation and differentiation of T-lymphocytes (Sazawal et al. 1997). Recent work has made it apparent that, thymic epithelial cells are the source of some peptides and hormones that influence T-cell maturation (Yan et al). Once T lymphocytes leave the thymus, their differentiation and maturation are regulated by zinc-thymulin (Hojyo and Fukada 2016). Thymulin is a Nano peptide produced by epithelial of the thymus. The hormone is involved in T-cell differentiation and enhancement of T and NK cell actions (Yan et al. 2017a). Thymulin activity necessarily is dependent upon the presence of the Zinc molecule being present in the thymulin peptide structure (Hojyo and

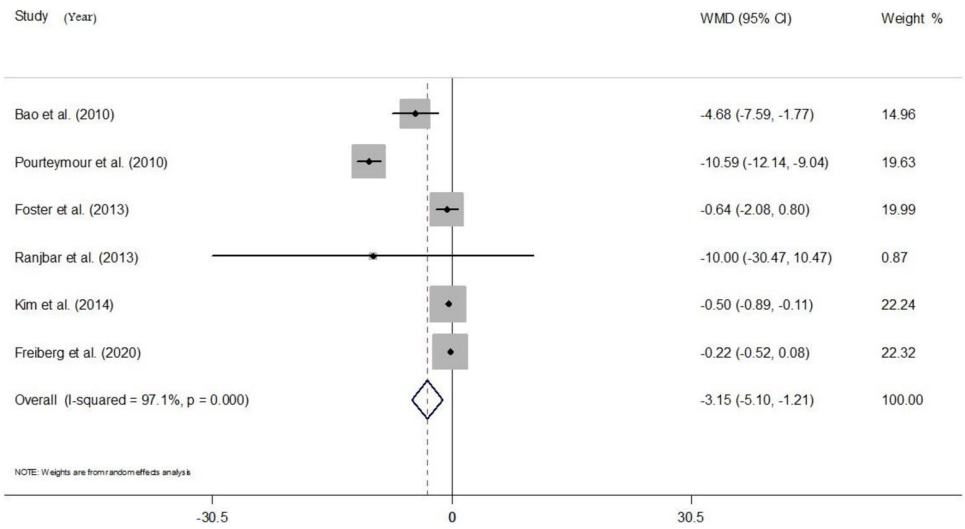

Figure 10. Forest plot of the effect of zinc on IL-6 using a random-effects model

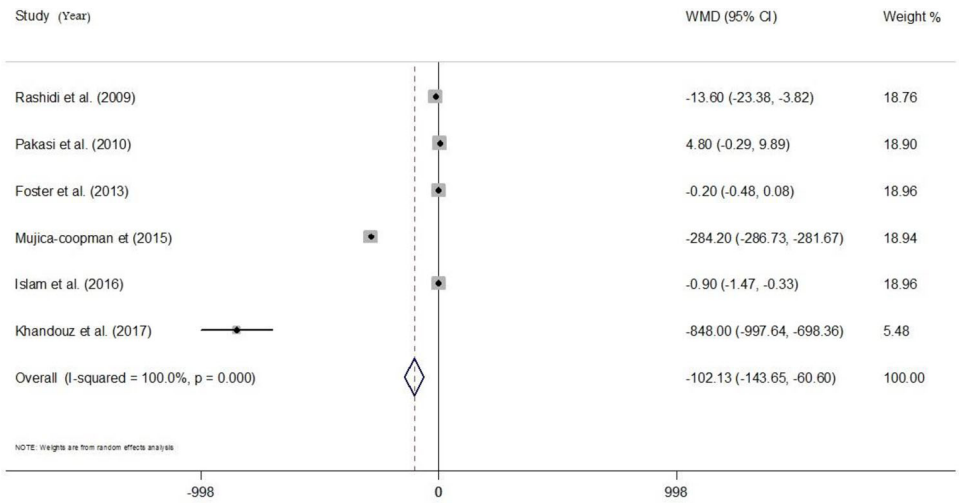

Figure 11. Forest plot of the effect of zinc on CRP using a random-effects model.

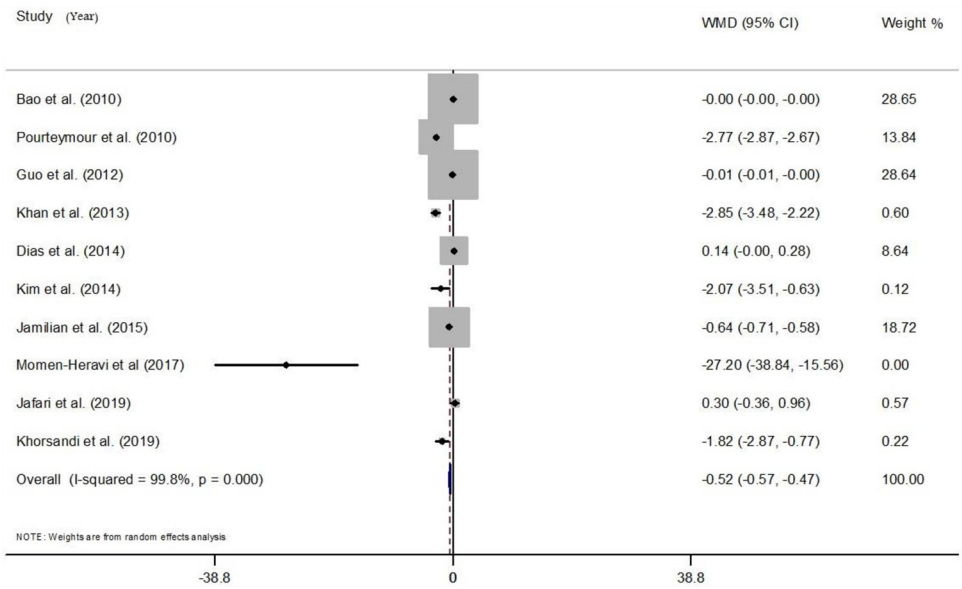

Figure 12. Forest plot of the effect of zinc on hs-CRP using a random-effects model.

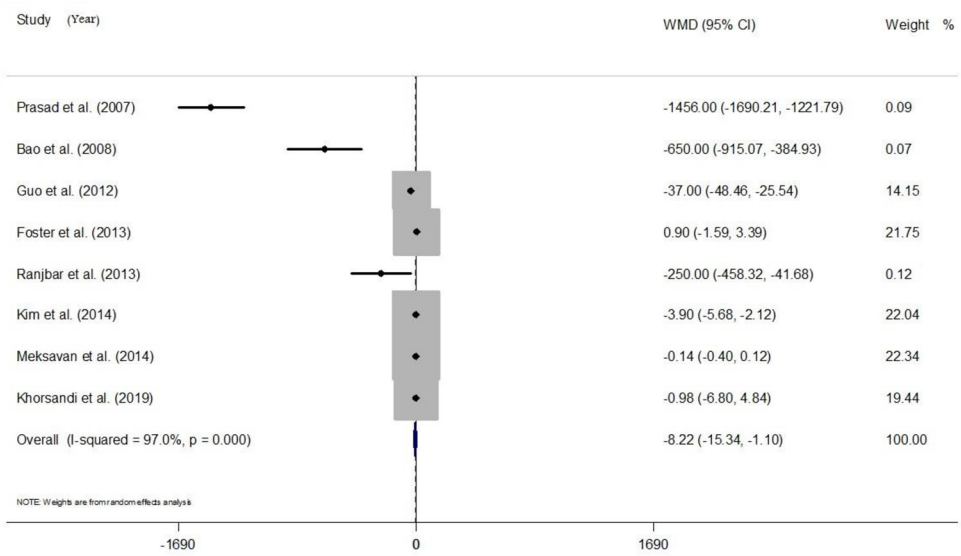

Figure 13. Forest plot of the effect of zinc on TNF-α using a random-effects model.

Fukada 2016). Thus possible mechanisms by which zinc may affect the T-cell development and function are through thymulin.

In the Zinc supplemented group, we failed to establish a significant increase in the CD4/CD8 ratio. These findings were also similar to other studies result (Bonomini et al. 1993; Raska et al. 1983). However, Holtkamp et al. (Holtkamp et al. 1991) reported that after zinc supplementation, the CD4/CD8 ratio was increased. Bonomini et al. (Bonomini et al. 1993) reported a significant reduction in CD8 cell percentages that occurred six months after Zinc supplementation. It is thought that Baseline zinc status, dosage, and duration affect immunity. Lower doses of Zinc (15 mg/d) significantly increased the ratio of CD4 to CD8 T lymphocytes at month six, and excessive dosage did not have significant long-term effects on immune status (Hodkinson et al. 2007). Zinc intake in the dosage of less than 15 mg/d seems to have not a positive impact on the immune response. On the other hand, excessive zinc intake impairs immunity (Chandra 1984). A significant increase in the CD4/CD8 ratio in hemodialysis (HD) patients was seen in the present review. We should consider that hemodialysis patients had low serum zinc levels at baseline, and the improvement in CD4/CD8 ratio is thought to be due to the repletion of immune impairment following zinc deficiency. So zinc deficiency in the participants highly affected the results.

We did not achieve a significant effect of zinc supplementation on WBC because of the short duration of studies that measured the WBC. Moreover, there was a high heterogeneity in the duration of studies ranged from 8 weeks to 6 months, therefore; we couldn't achieve a real result in this manner. However, in zinc-deficient and cancer patients, serum WBC increased after zinc supplementation; radiation therapy contributes to low immunity, especially cell-mediated immunity (Kuzmenok et al. 2003). The possible mechanisms responsible for the radiation-induced reduction of lymphocytes can be attributed to apoptosis. Also, the indirect mechanism was the radiation-induced expression of the lymphotoxins TNF-a, and CD95L both of which have been shown to have cytotoxic effects on lymphocytes (Veeraraghavan et al. 2011).

We also did not achieve a significant increase in lymphocyte and monocyte levels. We faced high heterogeneity between studies that measured lymphocyte, monocyte, and neutrophil as outcomes. The negative findings seem to be due to different study designs, different dosages, the form of zinc administration, and the duration of the studies.

The other marker of the immune state is CRP and hs-CRP. Among inflammatory markers, CRP has been examined its relation to dietary zinc intake among US healthy populations reporting a significantly positive relation to CRP levels (DE Oliveira Otto et al. 2011), and to serum zinc status among elderly Brazilian populations, which showed an inverse relationship (DE Paula et al. 2014). A significant inverse effect on CRP is seen in our study and surprisingly, the result was not dose-dependent. Moreover, Bao et al. noted that zinc supplementation reduced inflammatory biomarkers such as CRP in human subjects (Bao et al. 2010a) and Kim and Ahn et al. demonstrated that taking 30 mg/day supplemental zinc as zinc gluconate for eight weeks among young obese women decreased hs-CRP (Kim and Ahn 2014) levels and in the elderly subjects have been reported before (Kahmann et al. 2008). Additionally, the same positive result was seen in the study with HD patients (Roozbeh et al. 2011). Regarding the anti-inflammatory effect of zinc, it regulates the nuclear transcription factor (NF)-κB activation via anti-inflammatory proteins A20 and peroxisome proliferator-activated receptor (PPAR)-α signaling pathway (Jarosz et al. 2017). NF-κB, as a component of the adhesion molecule up-regulation process, increases CRP and inflammatory cytokines such as IL-1β and TNF-α (Jarosz et al. 2017; Aboonabi and Aboonabi 2020; Mussbacher et al. 2019). In cell culture studies, zinc decreased the generation of TNF-α, IL-1β, and the activation of NF-κB and increased the expression of A20 and PPAR-α in zinc sufficient human monocytic cells and vascular endothelial cells compared with zinc-deficient cells (Jarosz et al. 2017). So Zinc may enhance

or inhibit the activation of NF-κB depending on the zinc status of subjects. In vivo, diet-induced zinc deficiency in a mice model enhanced NF-κB activation in vital organs and the expression of a range of NF-κB targeted genes such as IL-1β and TNF-α, and short term zinc repletion before the onset of sepsis significantly reduced these effects through inhibition of NF-κB signaling (Bao et al. 2010c). More recently, it was discovered that zinc induces a specific cytokine response in humans; Zinc stimulated mononuclear cells in a dose-dependent manner to release IL-1, IL-6, and IFN-γ. IL-1, IL-6, and TNF-α are directly induced in monocytes by zinc (Prasad 2014b). This result is consistent with Costarelli et al. findings in which negative correlations between dietary zinc intake and inflammatory markers such as IL-6 and IL-1β were reported (Costarelli et al. 2008). So the decreased level of the plasma IL6 concentrations in the present survey can be interpreted by such mechanism.

We were faced with some important limitations in this review. First, the included studies were highly heterogeneous regarding intervention duration, the supplement's dosage, etc. Second, the types of dietary patterns which affect the subjects zinc level (usual diet, vegetarian, Mediterranean-like diet, etc.), and the health status of participants (healthy subjects, postmenopausal women, patients with type 2 diabetes, multiple sclerosis, ischemic heart disease, and metabolic syndrome) was not reported in most of the studies. The strengths of the present review and meta-analysis include our systematic protocol and comprehensive literature review approach, thus minimizing the possibility that any major published report was missed. This is the first attempt, to our knowledge, to survey the effect of zinc on immune responses. We also selected most of the immune markers and included both innate and adaptive immune response factors as an outcome. Moreover, the inclusion of manuscripts reporting controlled trials, with all being randomized, minimizes bias due to confounding at baseline and enhances the causal interpretation findings.

Conclusion

In conclusion, the present review showed that zinc supplementation is associated with a higher level of CD3, CD4, and CD8. Zinc supplementation also reduced CRP and hs-CRP levels significantly. We revealed that subjects' zinc status is associated with different immune responses as it was seen about WBC and CD4/CD8. These findings suggest that zinc status may need to be considered as a continuous variable when thinking about the immune systems role, rather than as a discrete variable where status is either deficient or adequate.

Acknowledgements

We want to thank S. Shahinfar and MR. Amini for their assistance.

Contributions of authors

AJ and ED designed the study. MA and AJ independently carried out the literature search and screening of articles; ED analyzed the data; AJ, ZN, and MA wrote the manuscript, and ED helped edit the writing. All authors read and approved the final manuscript.

Disclosure statement

The authors declare that they have no conflict of interest.

Funding source

This study has no funding.

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