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Profiling inflammatory cytokines following zinc supplementation: a systematic review and meta-analysis of controlled trials

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Abstract

Chronic inflammation has been considered as the main cause of chronic diseases. Zn has anti-inflammatory effects by decreasing the expression of inflammatory markers. The present systematic review and meta-analysis study aims to evaluate the impact of Zn supplementation on inflammation. PubMed (Medline), Scopus, Web of Science, and Embase databases were searched up to 10 December 2020. Controlled trials which have investigated the effects of Zn supplementation on serum/plasma levels of inflammatory cytokines in subjects aged >15 years were included. A pooled meta-analysis was performed using a random effect model. Sensitivity analysis was performed to determine the robustness of the observed effect sizes. A total of twelve studies was included in meta-analysis. Zn could decrease IL-6 levels (standardised mean difference (SMD) = −0·76 pg/ml; 95 % CI −1·28, −0·24; $P = 0·004$). There was no significant change in TNF-α (SMD = 0·42 pg/ml; 95 % CI −0·31, 1·16; $P = 0·257$) and IL-2 levels (SMD = 1·64 pg/ml; 95 % CI −1·31, 4·59; $P = 0·277$) following Zn supplementation. However, Zn could increase IL-2 significantly after the deletion of one arm in sensitivity analysis (SMD = 2·96 pg/ml; 95 % CI 2·03, 3·88; $P < 0·05$). Conclusively, Zn supplementation can decrease the IL-6 level. Zn increased IL-2 level after the sensitivity analysis. Zn supplementation has not ameliorative effects on TNF-α.

Key words: **Zinc: Inflammation: NF-κB: Systematic reviews: Meta-analyses**

Inflammation is a multifactorial network of chemical signals in response to detrimental insults such as tissue injury, other noxious conditions and microbial infection. It is a critical immune response by the host that removes the harmful stimuli as well as the healing of the damaged tissue[1]. Normal inflammation usually limits itself; however, dysregulation in any inflammatory factors can lead to abnormalities and pathogenesis. Systematic inflammation is believed to be associated with the initiation and progression of various chronic conditions such as cerebrovascular disease, dementia, ischemic heart and respiratory disease[2]. Also, the inflammatory response plays a vital role in different stages of tumour development, including initiation, progression, conversion to malignant and metastasis[3].

The inflammatory processes involve activation of the immune system, directed migration of leucocytes, and release of pro-inflammatory cytokines and mediators[4]. Cytokines are molecules with glycoprotein or protein structure that affect interactions and communication between cells[5]. Among the various type of cytokines, TNF-α, IL-2, IL-6 and IL-1 are significant

Abbreviations: SMD, standardised mean difference; TF, transcription factor.

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inducers of the acute-phase response[6,7]. TNF-α through binding to its receptor (TNFR1 and TNFR2) regulates the cytokine cascade in inflammatory pathways as well as cell proliferation, survival, differentiation and apoptosis of immune cells[8]. IL-2 acts as both inflammatory and anti-inflammatory agent via binding to its receptor, and regulation of these dual effects is important in the treatment of many inflammatory diseases[9]. Membrane IL-6 receptor (mIL-6 R) mediates IL-6 actions including the differentiation and maturation of immune cells and the induction of acute-phase protein synthesis in hepatocytes[10].

Production of these cytokines is mediated by different transcription factors (TF) including NF-κB, CCAAT/enhancer-binding protein (C/EBP)-β, the activator protein 1 (AP-1) and the nuclear factor of activated T cells[11–14]. Zn, as a trace element, plays an important role in the stabilisation of TF through Zn finger proteins[15]. A number of *in vivo* and *in vitro* studies have demonstrated that Zn can be effective in the regulation of mentioned TF, whereby it regulates inflammatory cascades[16–19].

Despite the number of trials which have investigated the effect of Zn supplementation on diseases associated with ongoing inflammation[20,21] as well as diabetes[22] and atherosclerosis[23], there is no comprehensive systematic review and meta-analysis to obtain a conclusive finding on the impact of Zn supplementation on cytokines. Therefore, this systematic review and meta-analysis study is conducted to evaluate the potential anti-inflammatory effects of Zn supplementation.

Material and methods

Search strategy

This systematic review and meta-analysis study was performed and reported in accordance with the guiding principle and recommendation of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA)[24]. PubMed (Medline), Scopus, Web of Science and Embase databases were searched up to 10 December 2020 by three authors (M. Z., Y. K. H. and A. H. F.) independently with no limitation for year and language. Search was performed by using following search pattern: ('Zinc' [Mesh] OR zinc[Title/Abstract]) **AND** (Interleukin-8[Title/Abstract] OR IL-8[Title/Abstract] OR CXCL8[Title/Abstract] OR NAP[Title/Abstract] OR NAP1[Title/Abstract] OR IL-2[Title/Abstract] OR interleukin-2[Title/Abstract] OR Interleukin-1[Title/Abstract] OR IL-1[Title/Abstract] OR Interleukin-1beta[Title/Abstract] OR Interleukin-1β[Title/Abstract] OR IL-1 β [Title/Abstract] OR IL-1beta[Title/Abstract] OR 'Interleukin-2' [Mesh] OR 'Interleukin-1beta' [Mesh] OR 'Interleukin-8' [Mesh] OR 'Tumor Necrosis Factor-alpha' [Mesh] OR tumor necrosis factor-alpha[Title/Abstract] OR TNF[Title/Abstract] OR 'tumor necrosis factor' [Title/Abstract] OR 'tumor necrosis factor-α' [Title/Abstract] OR tnf-alpha[Title/Abstract] OR tnf- α[Title/Abstract] OR tnfα[Title/Abstract] OR Tumour necrosis factor[Title/Abstract] OR 'Interleukin-6' [Mesh] OR interleukin-6[Title/Abstract] OR IL-6[Title/Abstract] OR interleukin6[Title/Abstract] OR IL6[Title/Abstract] OR inflammation[Title/Abstract]) **AND** (randomized controlled trial[Publication Type] OR controlled clinical trial[Publication Type] OR 'clinical trial' [Title/Abstract] OR random * [Title/Abstract] OR supplementation[Title/Abstract] OR placebo[Title/Abstract] OR groups[Title/Abstract] OR trial[Title/Abstract] OR 'randomized controlled trial' [Title/Abstract] OR 'controlled clinical trial' [Title/Abstract]. We used the wild-card term '*' to increase the search sensitivity.

Study selection and inclusion and exclusion criteria

After removing duplicate records, the titles and abstracts of the searched studies were screened based on the inclusion and exclusion criteria by three authors (O. M. T. R., E. F. and M. A.) independently. Controlled trials with parallel or cross-over design which have investigated the effects of Zn supplementation on the inflammatory cytokines were included. The PICO strategy for the research question of the study was Patient/Population (P): subjects with age of ≥15 years; Intervention (I): oral supplementation with Zn; Comparison (C): placebo or control group; and Outcome (O): changed TNF-α, IL-2 or IL-6 serum/plasma levels. Exclusion criteria were considered as follows: (i) other types of studies (*in vitro*, *in vivo*, *ex vivo*, quasi-experimental, reviews, letters, conference abstracts, case reports and observational studies), (ii) Zn supplementation along with another ingredient, (iii) infants and juvenile subjects, and (iv) lack of adequate information. Also, the reference lists of included studies were evaluated for other potential articles.

Data extraction

Articles meeting the inclusion criteria were abstracted by two authors (A. H. F. and L. P.) independently on the following items: first author and year of publication, journal, the region of investigation, sample size in each group, sex of subjects, mean age of subjects in each group, administered dosage and form of Zn, duration of treatment, and serum/plasma levels of inflammatory markers before and after the trial in each group. Any disagreements were discussed and resolved with a third reviewer (B. A.).

Study quality assessment

The Cochrane Collaboration tool was used for quality assessment of each trial included by two authors[25]. This tool assesses random sequence generation, allocation concealment details, blinding, elucidating of dropouts, reporting bias and other possible causes of bias. Each parameter was reported as high (−) or low (+) risk of bias, and unclear (?).

Statistical analysis

All data in included studies were reported as mean ± standard deviation. Reported means and standard errors, medians and ranges, and medians and interquartile ranges (Q25–Q75) were converted to means and standard deviations with statistical calculations. Pooled effect sizes were calculated using a random effect model with restricted maximum likelihood method. Because of the homogenised measurement units, the pooled effect sizes were expressed as standardised mean differences (SMD) with 95 % CI. I^2 statistic was used for the assessment of between-study heterogeneity and $I^2 > 50$ % was reported as high heterogeneity[26]. Potential sources of heterogeneity were identified using meta-regression and subgroup analysis based on sex, administered Zn dosage and form, duration, study population,

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and mean age. In meta-regression analysis, any linear relationship between the effect sizes and intervention duration, dose, and sample size were assessed. The influence of single study removal on the pooled effect size was assessed using sensitivity analysis. Publication bias was assessed using visual inspection of the funnel plot. In the presence of funnel plot's asymmetry, the trim and fill method was carried out for adjusting the results with estimating the missing studies that might exist in pooled analysis and the effect of these studies on the outcome. Small-study effects were investigated using Begg's adjusted rank correlation and Egger's regression asymmetry tests[27,28]. In all tests, $P < 0{\cdot}05$ was considered as significant level. All statistical analyses were performed using STATA version 16.0 (Stata Corporation).

Results

Study selection

Totally, 10 676 articles were obtained from a search in databases. Titles and abstracts of 7545 studies were screened after eliminating duplicate articles. Among which, 178 articles were eligible for assessing full texts. Finally, a total of twelve articles met the inclusion criteria and included in the quantitative synthesis. PRISMA flow chart for literature search and selection is presented in Fig. 1.

Characteristics of selected studies

Among included studies, eight studies with nine treatment arms, eight studies with eight treatment arms and two studies with three treatment arms investigated the effect of Zn supplementation on TNF-α, IL-6 and IL-2 levels, respectively. Four of the included studies were performed in Iran[29–32]. Other studies have been carried out in Turkey[33], Brazil[34], Russia[35], Australia[36], Thailand[37], USA[38] South Korea[39] and Poland[40] with a total sample size of 680 (varied from 17 to 254 participants). The included studies were performed from 2010 to 2020. The mean age of subjects ranged between 15 and 67 years. The Zn salts gluconate and sulphate were used in the included studies which provided 12–50 mg/d of elemental Zn. The duration of supplementation was between 4 and 72 weeks. Participants of four studies were chosen from females and others from both sexes. Inflammatory cytokines in five and seven studies were measured in serum and plasma, respectively. Characteristics of included studies are outlined in Table 1.

Risk of bias assessment

The results of Cochrane Collaboration's tool on the assessment of the risk of bias are presented in Fig. 2. As shown, random allocation was not observed in one included study. Moreover, the intention to treat protocol for analysing data in trials was not performed in four included studies. Four included studies had higher quality compared with others.

Effects of zinc on TNF-α

Zn supplementation had not a significant effect on TNF-α level in pooled estimate (SMD = 0·42 pg/ml; 95 % CI −0·31, 1·16; $P = 0{\cdot}257$) (Fig. 3(a)). This result was confirmed by sensitivity analysis. However, the gluconate form of Zn supplement led to a significant decrease in TNF-α level following subgroup analysis (SMD = −0·89 pg/ml; 95 % CI −1·35, −0·43; $P < 0{\cdot}001$) (Table 2). Moreover, TNF-α reduction was shown following <40 mg/d elemental Zn supplementation (SMD = −0·37 pg/ml; 95 % CI −0·74, −0·00; $P = 0{\cdot}048$). However, ≥40 mg/d elemental Zn supplementation resulted in a significant increase in TNF-α serum/plasma level (SMD = 2·73 pg/ml; 95 % CI 0·08, 5·39; $P = 0{\cdot}044$). There was a significant between-study heterogeneity ($I^2 = 87{\cdot}9$ %, $P < 0{\cdot}001$), which was reduced with subgrouping by the mean age of participants, Zn salt, study population and duration (Table 2). Meta-regression analysis showed that the effect size had no significant relationship with duration, mean age and sample size. Begg's test did not confirm the presence of a small-study effect ($P = 0{\cdot}118$). However, a significant small-study effect was shown following Egger's test ($P = 0{\cdot}02$). Moreover, visual inspection showed an asymmetric distribution in the funnel plot (Fig. 3(b)). However, trim and fill analysis confirmed the observed result (SMD = 0·98 pg/ml; 95 % CI −1·37, 3·34; $P > 0{\cdot}05$).

Effects of zinc on IL-2

Pooled analysis revealed that Zn supplementation had no significant effect on IL-2 level (SMD = 1·64 pg/ml; 95 % CI −1·31, 4·59; $P = 0{\cdot}277$) (Fig. 4(a)). However, sensitivity analysis revealed that removing Beserra de Moura *et al.* study[34] in pooled analysis led to a significant increase in IL-2 level following Zn supplementation (SMD = 2·96 pg/ml; 95 % CI 2·03, 3·88; $P < 0{\cdot}05$). Because of limited number of studies on IL-2, subgroup analysis and meta-regression were not possible. Egger's test but not Begg's test showed publication bias due to small-study effects ($P = 0{\cdot}024$ and $P = 0{\cdot}296$, respectively). Moreover, visual inspection of the funnel plot illustrated the asymmetric distribution of studies (Fig. 4(b)). However, trim and fill analysis with three observed studies confirmed the observed results (SMD = 1·55 pg/ml; 95 % CI −0·97, 4·08; $P > 0{\cdot}05$).

Effects of zinc on IL-6

The results of forest plot showed that Zn supplementation could decrease IL-6 level (SMD = −0·76 pg/ml; 95 % CI −1·28, −0·24; $P = 0{\cdot}004$) (Fig. 5(a)). Duration of supplementation, study population and administered dosage were recognised as sources of observed high between-study heterogeneity ($I^2 = 85{\cdot}1$ %, $P < 0{\cdot}001$) (Table 2). Also, subgroup analysis revealed that a higher dosage of Zn supplements (≥40 mg/d), female sex, healthy population and higher mean age (>40 years) resulted in a more reduction of IL-6 levels (Table 2). Among Zn forms, zinc gluconate had an ameliorative effect on the IL-6 level (Table 2). Meta-regression analysis revealed no significant relationship between the effect size and intervention duration, dose, and sample size. There were no small-study effects following Egger's and Begg's tests ($P = 0{\cdot}181$ and 0·108, respectively). Moreover, symmetric distribution of studies was visualised in the funnel plot (Fig. 5(b)).

Discussion

As our knowledge, this systematic review and meta-analysis study is the first comprehensive investigation evaluated the possible effects of Zn supplementation on serum/plasma profile of inflammatory cytokines including IL-2, IL-6 and TNF-α using published

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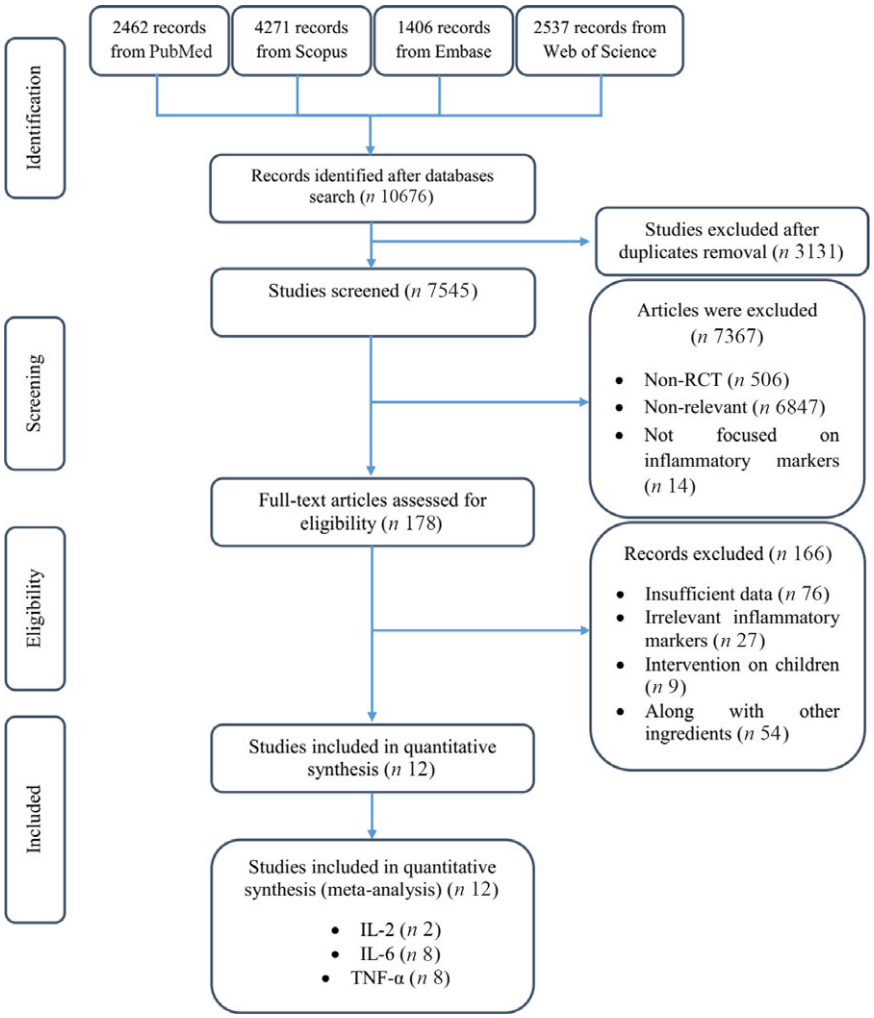

Fig. 1. The process of study selection through stages shown by Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) flow chart. RCT, randomised controlled trial.

trial data. We revealed that Zn supplementation had an ameliorative effect on the IL-6 level. Zn forms, dosage, intervention duration, study population and mean age of participants could affect the pooled estimate and considered as the potential sources of heterogeneity. We did not include C-reactive protein (CRP) level in our meta-analysis since other meta-analysis study by Mousavi *et al.* revealed that Zn supplementation could decrease CRP level[41].

We demonstrated that Zn supplementation had no ameliorative effect on TNF-α level following pooled analysis of nine relevant studies. Subgroup analysis revealed that intervention duration, study population, mean age of participants and Zn forms of the supplement were potential sources of high heterogeneity. Duration of supplementation and the mean age of participants were from a broad range, and these could cause various results. Analysis of two studies that administered the gluconate form revealed that zinc gluconate had a beneficial effect on the TNF-α level. One possible cause is difference in bioavailability. Sapota *et al.* revealed that the rats administered zinc sulphate had the lowest Zn in the prostate tissue compared with those supplemented with zinc gluconate[42]. On the other hand, another *in vivo* study reported that zinc gluconate and zinc sulphate had equivalent bioavailability[43]. The second cause is study population; in one study administered zinc gluconate, subjects had Zn deficiency[34]. It seems Zn supplementation had a more beneficial effect on inflammatory markers in the Zn deficiency status. In the other study, subjects were obese persons who had altered Zn redistribution[39]. Surprisingly, Zn supplementation increased TNF-α level in higher dosages (≥40 mg/d elemental Zn) subgroup and decreased TNF-α level in lower dosage (<40 mg/d elemental Zn). The tolerable upper intake level of Zn for adults has been determined 40 mg/d[44]. Zn acts as a pro-oxidant agent in overload condition through disrupting of mitochondria homoeostasis and excessive reactive oxygen species production[45]. Moreover, Zn toxicity might be related to increase in pro-inflammatory cytokine production[46].

We failed to find a significant association between Zn supplementation and the IL-2 production in the pooled estimate. However, sensitivity analysis revealed that Zn supplementation led to a significant increase in IL-2 level after omitting Beserra de Moura *et al.* study[34]. The intervention groups in the mentioned

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Table 1. Characteristics and baseline measurements of included studies (Mean values and standard deviations)

First author	Location	Year	Patient features	Sex	Sample size (*n*)	INT mean age* (years)		CONT mean age* (years)		Duration (weeks)	Measurement location	Zn salt	Elemental Zn (mg/d)	Baseline serum Zn (μg/dl)	Main outcomes
						Mean	SD	Mean	SD						
Meksawan[37]	Thailand	2014	Type-2 diabetic	NR	17	58·75	10·53	57·67	10·05	8	Plasma	Sulph	30	75·25	TNF-α (NS)
Kara[33]	Turkey	2011	Healthy (sportsmen)	F/M	20	15–17†		15–17†		8	Serum	Sulph	NR (5 mg/kg Zn salt)	NR	TNF-α ↑ IL-2 ↑
Kara[33]	Turkey	2011	Healthy (sedentary)	F/M	20	15–17†		15–17†		8	Serum	Sulph	NR (5 mg/kg Zn salt)	NR	TNF-α ↑ IL-2 ↑
Kim[39]	South Korea	2014	Healthy	F	40	20·8	2·2	20·8	2·2	8	Plasma	Gluc	30	84·99	TNF-α (NS) IL-6 ↓
Ranjbar[29]	Iran	2014	Major depression	F/M	37	37·0	9	37·5	8	12	Plasma	Sulph	25	NR	TNF-α (NS) IL-6 (NS)
Sulibursk[40]	Poland	2018	Hypertension	F/M	65	53·6	13·7	53·6	13·7	4	Serum	NR	15	59·5	TNF-α ↓
Foster[36]	Australia	2013	Type-2 diabetic	F	22	NR (PW)		NR (PW)		12	Plasma	Sulph	40	87·61	TNF-α (NS) IL-6 (NS)
Khorsandi[30]	Iran	2019	Obese subjects	F/M	40	35·63	3·2	32·95	1·7	15	Plasma	Sulph	30	65·2	TNF-α (NS)
Beserra de Moura[34]	Brazil	2020	Ulcerative colitis	F/M	41	47·22	11·8	45·33	12·4	8	Serum	Gluc	35	53·2	TNF-α (NS) IL-6 (NS) IL-2 ↓
Bao[38]	USA	2010	Healthy	F/M	40	65	7	67	7	24	Plasma	Gluc	45	600·84	IL-6 ↓
Pourteymour Fard Tabrizi[31]	Iran	2010	PCOS	F	60	27·17	4·58	26·93	4·77	8	Serum	Sulph	50	76·11	IL-6 ↓
Roshanravan[32]	Iran	2017	Pregnant women with IGT	F	44	29·45	4·21	29·82	5·41	8	Serum	Gluc	30	NR	IL-6 ↓
Freiberg[35]	Russia	2020	HIV heavy drinker	F/M	254	34	5	34	6	72	Plasma	Gluc	15 M/12 F	NR	IL-6 (NS)

INT, intervention group; CONT, control group; NR, not reported; Sulph, sulphate; Gluc, gluconate; F, female; M, male; PW, postmenopausal women; PCOS, polycystic ovary syndrome; IGT, impaired glucose tolerance.
* From the same studies with investigating Zn supplementation on different subjects.
† Expressed as range.

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Fig. 2. The result of risk of bias assessment using Cochrane Collaboration's risk of bias tool: each risk of bias item for included studies (green (+) means low risk of bias, yellow (?) means unclear risk of bias, red (–) means high risk of bias).

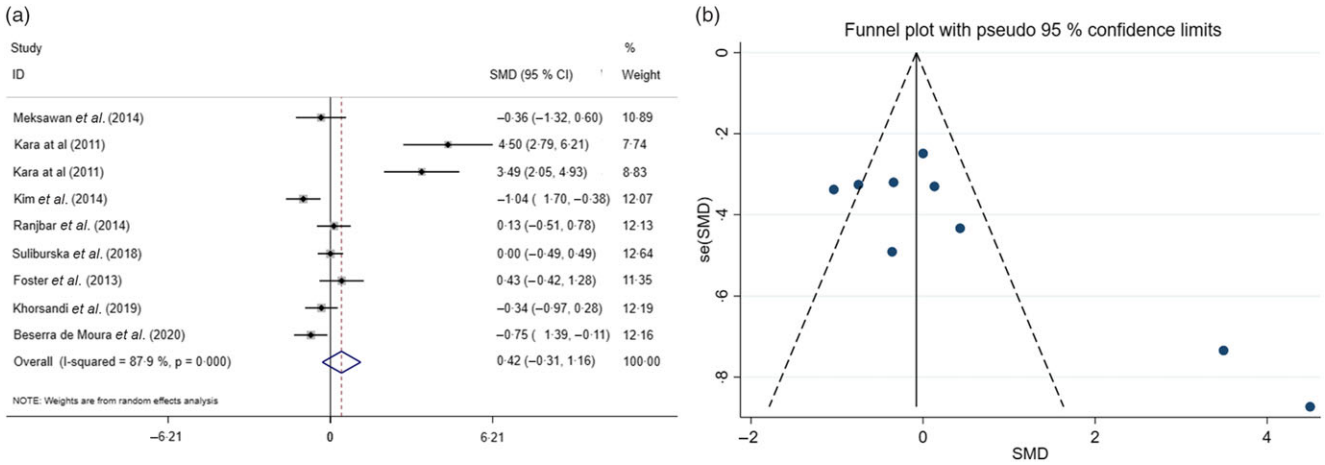

Fig. 3. Forest plot (a) detailing standardised mean differences (SMD) and 95 % confidence intervals and funnel plot (b) displaying publication bias in the studies reporting the effects of zinc supplementation on TNF-α level.

study were subjects with Zn deficiency in whom their serum Zn levels were not raised enough to reach Zn sufficiency status during the supplementation, likely because of short duration and low dosage of intervention. Since IL-2 production is impaired in Zn-deficient subjects[47], their IL-2 levels remained low. IL-2 reduction in Zn deficiency status is related to the expression of the TF cAMP-responsive element modulator α (CREMα) which is involved in IL-2 transcription. CREMα binding site is cAMP-responsive element which is located within the IL-2 promoter. Therefore, its high expression inhibits IL-2 transcription[48]. *In vitro* investigation revealed that Zn deficiency increased CREMα expression[48].

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Table 2. Pooled estimates of effects of zinc on inflammatory markers within different subgroups (Standard mean differences (SMD) and 95 % confidence intervals)

	Group	No. of comparisons	SMD	95 % CI	*P*	I^2 (%)	*P*-heterogeneity
TNF-α	Total	9	0·42	−0·31, 1·16	0·257	87·9	<0·001
	Elemental Zn dosage (mg/d)						
	<40	6	−0·37	−0·74, 0·00	0·048	48·8	0·082
	≥40	3	2·73	0·08, 5·39	0·044	91·9	<0·001
	Intervention duration (weeks)						
	≤8	6	0·75	−0·43, 1·93	0·214	92·2	<0·001
	≥12	3	0·01	−0·41, 0·44	0·949	12·9	0·317
	Mean age (years)						
	<40	5	1·15	−0·32, 2·61	0·125	93·3	<0·001
	>40	4	−0·18	−0·67, 0·3	0·459	47·5	0·127
	Sex						
	F	2	−0·33	−1·77, 1·11	0·654	86	0·008
	F/M	6	0·89	−0·14, 1·91	0·089	91	<0·001
	NR	1	−0·36	−1·32, 0·6	0·463	—	—
	Zn salts						
	Gluconate	2	−0·89	−1·35, −0·43	<0·001	0·0	0·541
	Sulphate	6	1·11	−0·04, 2·26	0·059	89·6	<0·001
	NR	1	0·00	−0·49, 0·49	1·00	—	—
	Study population						
	Healthy	3	2·26	−1·57, 6·1	0·247	96·5	<0·001
	Unhealthy	6	−0·16	−0·48, 0·16	0·333	26·8	0·233
IL-6	Total	8	−0·76	−1·28, −0·24	0·004	85·1	<0·001
	Elemental Zn dosage (mg/d)						
	<40	5	−0·29	−0·48, −0·1	0·003	0·0	0·806
	≥40	3	−1·49	−2·68, −0·3	0·014	87·1	<0·001
	Intervention duration (weeks)						
	=8	4	−0·98	−2·00, 0·04	0·059	90·3	<0·001
	=12	2	−0·36	−0·87, 0·17	0·184	0·0	0·626
	≥24	2	−0·7	−1·72, 0·33	0·182	87·5	0·005
	Mean age (years)						
	<40	5	−0·8	−1·56, −0·04	0·038	90·4	<0·001
	>40	3	−0·7	−1·31, −0·09	0·025	54·7	0·11
	Sex						
	F	4	−1·05	−2·09, −0·00	0·049	89·1	<0·001
	F/M	4	−0·48	−0·89, −0·03	0·036	62·9	0·044
	Zn salts						
	Gluconate	5	−0·5	−0·85, −0·15	0·005	54·8	0·065
	Sulphate	3	−1·14	−2·68, 0·4	0·147	92·5	<0·001
	Study population						
	Healthy	2	−0·95	−1·56, −0·34	0·002	41·3	0·192
	Unhealthy	6	−0·7	−1·35, −0·05	0·036	88·0	0·192

F, female; M, male; NR, not recognised.

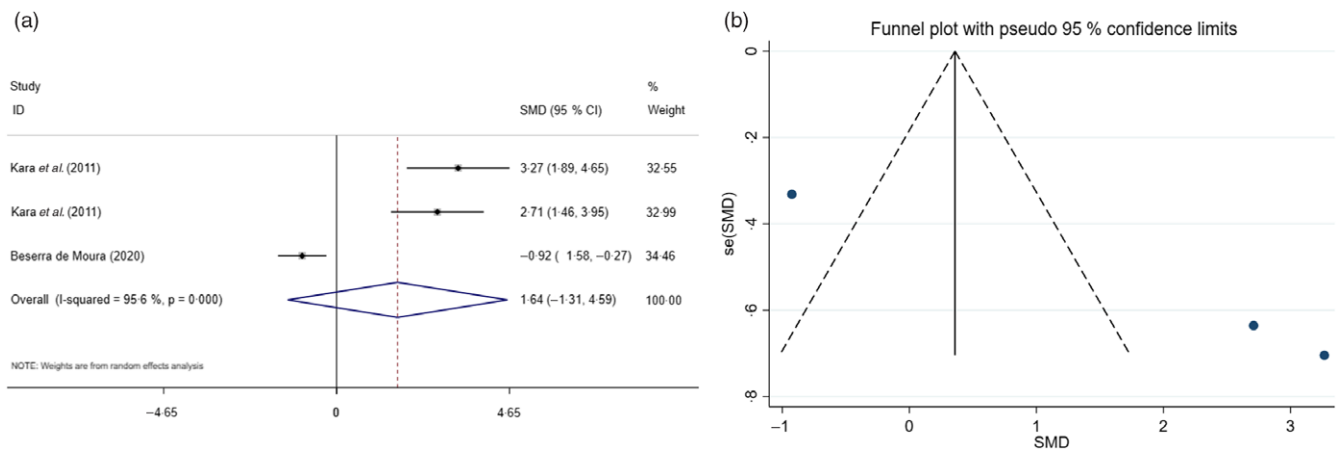

Fig. 4. Forest plot (a) detailing standardised mean differences (SMD) and 95 % confidence intervals and funnel plot (b) displaying publication bias in the studies reporting the effects of zinc supplementation on IL-2 level.

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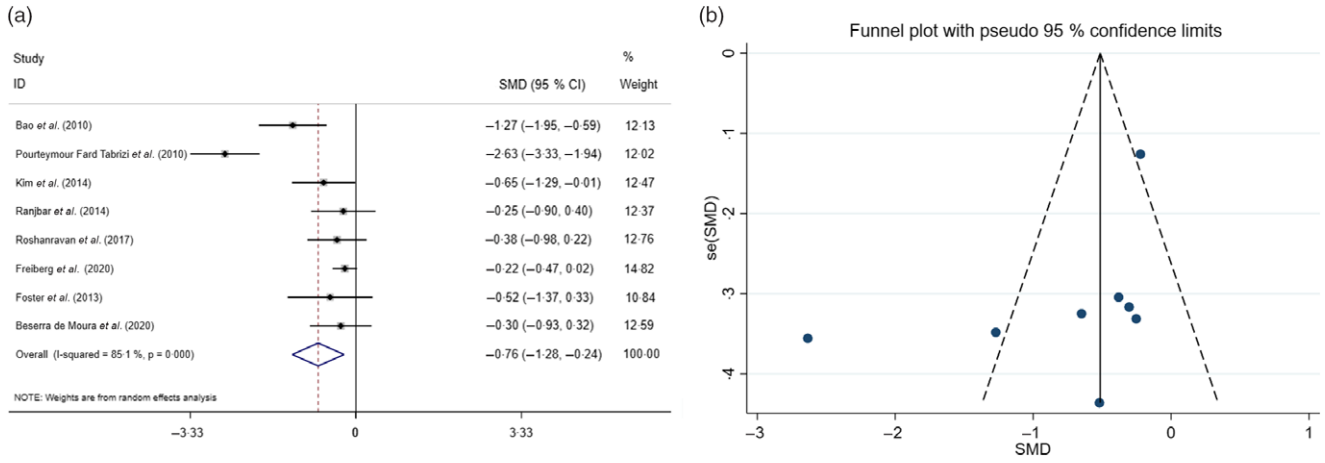

Fig. 5. Forest plot (a) detailing standardised mean differences (SMD) and 95 % confidence intervals and funnel plot (b) displaying publication bias in the studies reporting the effects of zinc supplementation on IL-6 level.

IL-2 has dual effects on inflammatory pathways. It is a promoter of T-cell proliferation and T-helper 1 (Th1) and Th2 effector cells generator and whereby induces inflammation. On the other hand, IL-2 inhibits the production of inflammatory Th17 cells and has a pivotal role in regulatory T-cell maintenance[49]. In lower dosage, IL-2 has been used as a therapeutic agent in the various types of cancer and inflammatory conditions because of its effect on immune system function and anti-inflammatory impacts in lower dosage[9,50]. However, in higher dosage, IL-2 may lead to toxicity and inflammation in some organs like skin and lung[50]. It is not known whether observed increase in IL-2 concentration in our meta-analysis has inflammatory effects or anti-inflammatory. It must be noted that participants of included studies in our meta-analysis on the effect of Zn treatment on IL-2 level were from people with a low concentration of serum/plasma Zn. Moreover, other *ex vivo* studies revealed that Zn supplementation had increasing effect on IL-2 production in Zn-deficient subjects[47,51]. Therefore, it seems that Zn supplementation corrected the low level of IL-2 as an anti-inflammatory agent.

Nine comparisons of eight relevant studies revealed that Zn supplementation could decrease the IL-6 level. Intervention duration, dosage, study population and Zn forms were potential sources of high heterogeneity. Similar to TNF-α pooled analysis, intervention duration and elemental Zn dosage were from a broad range: 12–50 mg/d and 8–72 weeks, thereby they could cause heterogeneity in the results. Higher dosage of elemental Zn (≥40 mg/d), gluconate form and older subgroups had more decrease of IL-6 serum/plasma level. As discussed above, more beneficial effect of gluconate form may be related to its better bioavailability. Elderly people are more at risk for chronic inflammation and oxidative stress than younger people[52]. As a result, Zn supplementation seems to have a more beneficial effect on inflammation in the elderly subjects than in the young. Higher dosage of Zn had different impacts on TNF-α and IL-6 levels. TNF-α is an early response cytokine[53,54]. Pro-oxidant conditions, caused by Zn overload, firstly elevate TNF-α level. It seems that a long-term supplementation with a high dosage of Zn is needed to have an increasing effect on IL-6 levels. The results on healthy and patient subjects were not very different, and more studies on people with different health statuses are needed to draw the right conclusion. Bao *et al.* study[38] was the only study with higher baseline plasma Zn. However, sensitivity analysis revealed that omission of mentioned study did not change the overall result.

Zn has a possible effect on IL-2, IL-6 and TNF-α levels through the regulation of NF-κB activation. Dimerisation of NF-κB mediated by other subunits like p50, p65, c-Rel and RelB is essential for its binding to DNA[55]. Zn finger proteins like A20, growth factor independence-1 (Gfi-1), Zn finger and BTB domain containing 20 (ZBTB20), and Zn finger protein-64 (ZFP-64) are involved in NF-κB signalling; as A20 and Gfi-1 inhibit NF-κB dimerisation; ZBTB20 and ZFP-64 promote NF-κB dimerisation[56]. It has been found that Zn supplementation induces A20 binding to mRNA and DNA via the upregulation of mRNA and DNA-specific sites and whereby acts its anti-inflammatory effect[52]. In an *in vivo* study, Zn was contributed to the reduction of NF-κB p65 mRNA expression in the jejunum of weaned piglets[57]. Moreover, stabilisation of other TF including CCAAT/enhancer-binding protein (C/EBP)-β, AP-1 and nuclear factor of activated T cells which are contributed in the production of cytokines is mediated by Zn. Zn status also can be effective in mRNA expression of a cytokine through binding to metal response elements on the promoter of target genes[58,59].

Some limitations of our study must be noted. First, the quality of some included studies was low. Random allocation and blinding of participants, researchers, and outcome assessments were observed only in six and four included studies, respectively. Second, present review was not registered in any registration databases. Third, since some studies had not determined the basic serum/plasma level of Zn in study population, subgroup analysis based on basic Zn level was not performed. Fourth, as study population of included studies were different, we used random effect model to control unobserved heterogeneity. However, random effect model does not generalise the results of the performed meta-analysis to real-world situations[60]. Fifth, because of limited number of studies on IL-2, subgroup analysis and meta-regression were not possible. Therefore, sources of heterogeneity were unknown and this question the

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generalisability of our result on IL-2. Sixth, we could not include studies on other inflammatory markers. There are a variety of inflammatory cytokines like IL-1 and IL-8 which we included in our search regimen. However, because of the limited number of studies on each, meta-analysis on them was impossible. Other trial studies on the other inflammatory markers are needed to find a comprehensive conclusion about the effect of Zn on the inflammatory process.

There are some strengths in our meta-analysis. First, it is the first comprehensive meta-analysis study which evaluated the effect of Zn supplementation on the inflammatory cytokines. Second, we analysed the included studies based on different subgroups to find a defined conclusion. Third, we assessed all included studies with appropriate tests to find any source of heterogeneity and bias between the studies.

Conclusion

Zn supplementation can decrease IL-6 serum/plasma level. Zn has no significant effects on IL-2 and TNF-α production. Lower dosage of zinc gluconate has ameliorative effect on TNF-α serum/plasma level. Dosage and form of Zn supplement are two key factors in the effectiveness of Zn supplementation in modifying inflammatory responses. A dosage lower than upper intake level of zinc gluconate seems to have a better effect on inflammatory markers.

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