Zinc in depression: From development to treatment: A comparative/ dose response meta-analysis of observational studies and randomized controlled trials

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ABSTRACT

Background: A previous meta-analysis suggested that zinc status may be linked to depression status. However, it remains unclear whether zinc status can predict the risk of depression development, or whether the monotherapy of zinc is superior to the combination of zinc supplementation and antidepressant medications in the treatment of depression. Therefore, this meta-analysis aimed to clarify the impact of zinc status and supplementation on depression development and status across all available evidence.

Methods: PubMed, EMBASE, Scopus, and ISI web of science were searched, up to 14 May 2020, for relevant publications. Pooled relative risks (RRs) with 95% confidence intervals (CI) in observational studies, and mean and standard deviation (SD) for the change in depression score in RCTs were calculated using a random-effects model.

Results: The meta-analysis of RCTs indicated that zinc supplementation significantly lowered depressive symptom scores of depressed patients [weighted mean difference (WMD = −4.15 point; 95% CI: −6.56, −1.75 point; $P < 0.01$)], and the improvement in depression status occurred only when zinc supplementation was prescribed as a monotherapy. The cohort studies showed that the highest level of zinc intake was associated with a 28% reduced risk of depression (RR: 0.66; 95% CI: 0.50, 0.82; $I^2 = 13.90$). Dose-response analyses revealed a significant non-linear effect of baseline mood status on depression score.

Conclusion: Current evidence from observational studies and RCT's supports the potential benefits zinc to reduce the risk of, and alleviate, depression. However, further trials are needed to confirm the beneficial effect of zinc as a monotherapy versus adjunctive therapies.

Keywords: Zinc, depression, monotherapy, meta-analysis,

INTRODUCTION

The monoamine hypothesis, accepted as the most common hypothesis with regard to the pathophysiology of depression [1, 2], has led to the development of almost all currently used antidepressant drugs [3], including selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs) [3, 4]. However, remission is achieved in only one-third of patients after treatment with SSRIs [3]. Antidepressants have latency of response [1, 5], indeed, some evidence suggests that the monoamine hypothesis may be inadequate and emphasizes the need for creating alternative, preventative and treatment, approaches to antidepressant medication [1, 4]. Micronutrients currently represent the most prominent and valid alternate to monoamine-based antidepressant medicine, as introduced in the "nutritional psychiatry hypothesis" [6, 7].

Zinc is a micronutrient to have received much attention, due to its' possible role in depression [8]; for instance, zinc dysregulation in the hippocampus, amygdala, and the cerebral cortex is purportedly linked to the pathophysiology of depression [9-13]. Furthermore, dysregulation of brain zinc status is reported in many psychiatric and neurological disorders, such as schizophrenia [14], mood disorders [15], Parkinson's [16], and Alzheimer's disease [16]. Regulation of zinc levels within the brain may have a critical therapeutic role in neuropsychiatric diseases [11]. Indeed, support for this hypothesis originates from studies' reporting that zinc deprivation can induce depressive-like behavior, which can be effectively reversed by zinc supplementation [17, 18]. Furthermore, it is conceivable that zinc could be used to enhance the antidepressant effects of drugs belonging to the SSRI group [19, 20]. Considering this viewpoint,

a connection between zinc and depression is highly probable. Concordantly, a meta-analysis of seventeen observational studies reported that serum zinc concentration was lower in depressive patients, as compared to a healthy population, whilst the severity of depression status was related to the degree of zinc deficiency [21]. A recent meta-analysis, by Li et al., reported an inverse association between zinc status and risk of depression; however, this study exclusively considered zinc intake [22]. Several meta-analyses have investigated the effect of zinc supplementation on depression status, however, these studies mainly focused on the efficacy adjunctive zinc therapy [23-25]. Notwithstanding the previous investigations, it is unclear whether the monotherapy of zinc is superior to the combination of zinc supplementation and antidepressant medications in depression, furthermore sources of heterogeneity are currently unclear. Therefore, this meta-analysis sought to clarify the impact of zinc status and supplementation on depression development and status across all available observational and RCTs, and to conduct a dose-response analysis to investigate whether the effect of zinc supplementation on depression symptoms had non-linear association.

Materials and Methods:

The present systematic review and meta-analysis was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26]. We also followed the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines for reporting the meta-analysis of observational studies [27]. The review protocol was registered with the Prospero International Prospective Register of Systematic Reviews (http://www.crd. york.ac.uk/PROSPERO registration number CRD42018108150)

Search strategy

PubMed, EMBASE, Scopus, and ISI web of science, were searched for relevant medical literature, without language or publication date restriction, up to 14 May2020. The keywords used in our

search strategy were “zinc” and “depression”. Further details about the search strategy in the aforementioned databases are provided in **Supplementary Table 1**. Reference lists of previous review papers that investigated the association between zinc and depression were also checked for any additional studies that were not identified by the database searches. All titles and abstracts were screened by two authors (SY and P Sh) to find eligible studies.

Inclusion criteria

All publications with cross-sectional, cohort, case-cohort, and nested case-control designs, as well as follow-ups of randomized controlled trials (RCTs**)** that described zinc-depression association, were included. RCTs, as well as observational studies, were included if; 1) they were original studies, 2) they reported depression score as outcome, 3) zinc was used as the intervention approach (RCTs) or exposure/risk factor in observational studies, 4) the study was conducted in a general adult population. If multiple studies were published on the same population, then the most recent, with most complete reports, were included. RCT studies that addressed the effect of zinc combined or compared with other vitamins and minerals were excluded. Studies were also excluded if they were conducted in lactating/pregnant women.

Data extraction

The following data from eligible studies were extracted: the name of first author, year of the publication, country, study design, number of participants, depression score and tools, sex, length of study or follow-up, type/dose of zinc used in the RCTs design and the method of zinc assessment in observational studies, use of antidepressant medication, and list of adjusted variables in observational studies. Data extraction and study selection were conducted independently by two investigators (SY, MA). In instances of divergence, a third reviewer (SS) was consulted.

Quality of evidence:

The Cochrane collaboration tool was used to assess the quality of the RCTs studies. This tool categorizes the quality of studies into two levels: low quality, defined as a Cochrane collaboration

score lower than five points, and high quality defined as a score ≥ 5 [28]. A modified version of the Newcastle Ottawa Scale (NOS), designed for nonrandomized studies, was used to quality assessment of the eligible observational studies [29]. This scale has a range of 0 to 9, where studies with scores equal 9 points represents the highest quality.

Statistical analysis

We conducted separate analyses for RCTs and observational studies, in order to address the fundamental differences between these types of study designs. For RCTs studies, our outcome of interest was the difference in pre to post-intervention changes in depression scores with control groups. If changes were not reported, the mean and SD of changes from baseline to follow-up were estimated for each intervention and control group using the calculated correlation coefficient for studies that reported the baseline, after follow-up, and change values [r=0.67] [20, 30, 31]. We calculated the mean difference and its corresponding standard error (SE) as the effect size to perform the meta-analysis, because the depression score values were reported in the same scales (Beck questionnaire). The random-effects model was used to pool the data from included studies when the extent of inconsistency (I^2) was > 50 % [32]. Sensitivity analyses were also conducted to evaluate the impact of individual studies on overall pooled estimates and heterogeneity. We evaluated the potential sources of heterogeneity with the following subgroup analyses: depression scale (Beck compared with Hamilton questioner), study duration (<12 weeks compared with ≥12 weeks), and adjunctive therapy (with or without anti-depressive agent therapy). Moreover, a meta- regression analysis was conducted to check the effect of age, baseline depression score, dose of zinc supplementation, and the study duration (as a continues form) on predicting WMD of depression status. The potential non-linear effects of the age of participants and baseline mood status on depression score was investigated using fractional polynomial models for RCTs [33] .

For observational studies, the meta-analysis was performed by combining the multivariable-adjusted odds ratios (ORs) and their 95% confidence intervals (95% CIs) for comparing the prevalence or the incidence of depression between groups with the highest and lowest zinc status. Relative risks (RRs) were considered as ORs when used in the meta-analysis. The effect size was pooled based on the random-effects model using the Der Simonian–Laird method, which incorporated both within and between-study variability [34]. Statistical heterogeneity was assessed using Cochran's Q test and I^2 statistic [35]. Publication bias was evaluated by Egger's regression asymmetry test [36] and Begg's adjusted rank correlation test [37]. The meta-analysis was performed with STATA software (version 13.0; Stata Corp), and P values less than 0.05 were considered statistically significant.

RESULTS

Literature search and study characteristics. A total of thirteen observational studies (9 cross-sectional studies and four cohort studies) [38-49], and eight RCTs [19, 20, 30, 31, 50-53] met the inclusion criteria for meta-analysis, from the 4245 articles initially retrieved from the electronic search. The selection process of included studies is detailed in **Supplementary Figure 1 and Supplementary Figure 2**.

RCT studies. RCT studies were conducted between 2003 and 2018 [19, 20, 30, 31, 50-53]. These studies provided data for 319 participants (intervention = 159, control= 160). Trial durations ranged from 2 to 12 weeks; all studies conducted in both sexes. Three RCT studies investigated the effect of zinc supplementation compared to placebo [51, 30, 31], whilst in five RCT studies, the adjunctive effect of zinc versus antidepressant drugs was evaluated [50, 19, 20, 52, 53]. Most RCTs were conducted in Iran [20, 30, 31, 50-52], and the remainder (*n*=2) were conducted in Poland [19, 53]. The participants were depressed-only [19, 20, 31, 50-53] in most included studies, except one study, which was conducted on a mixed population (depressed and non-depressed

subjects) [30]. The study by Sawada reported the effect of zinc supplementation compared to multivitamins, thus, they were not included in the meta-analysis of RCTs [54]. Finally, eight RCT studies were included in the final analysis (**Table 2)**. The non-linear dose-response analysis failed to detect any significant effect of participant's age on depression score (*P*-nonlinearity= 0.721) (**Supplemental Figure 3**), although there was a non-linear trend between baseline mood status on depression score in patients with mild to moderate depression (20-30 score) (**Supplemental Figure 4**).

Observational studies: Studies were published between 2012 and 2020, nine of which followed a cross-sectional design [38, 41, 40, 42, 43, 45-47, 49]; two were carried out in the USA [45, 46], five in Asia [38, 47, 40, 41, 49], one in Germany [43], and one in Australia [42]. From four prospective cohort studies, three studies were conducted in Australia [39, 48], and one in Finland [44]. The number of subjects and ages surveyed in the cross-sectional and prospective cohort studies ranged from 297 to 14834 (age: <18-79 years), and from 1705 to 9738 (age: from <18 to ≥70 year), respectively (**Table 1)**.

Finding's from RCTs. The seven meta-analyzed RCTs indicated a significantly greater reduction in depression score with the zinc supplementation than with the control diets [weighted mean difference (WMD = −4.15 point; 95% CI: −6.56, −1.75 point; P < 0.01)] (19, 20, 30, 31, 44-47), with substantial heterogeneity between studies (I^2 = 80.1%; P-heterogeneity <0.001)(**Figure 1**). Subgroup analyses revealed that zinc supplementation significantly reduced depression scores in depressive patients who received zinc supplementation in the absence of anti-depressant medications (**Table 3**). The results of four models of meta-regression are presented in **Table 4**. Age, dose of zinc supplementation, study duration, and baseline depression score, in each study, were negatively associated with WMD for absolute change of depression score.

The omission of each study, individually, from the meta-analysis did not alter the overall effects. In addition, the Egger's linear regression tests (P=0.393) and Begg's test (P=0.368) did not indicate evidence for potential publication bias (**Supplementary Figure 5**).

Findings from prospective cohort studies. Four cohort studies (15852 Participants, 2243 incidence of depression) investigated the association between high versus low zinc intake and risk of depression [39, 44, 48]. The highest level of zinc intake was associated with a 28% reduced risk of depression (RR: 0.66; 95% CI: 0.50, 0.82; I^2 = 13.90%; P-heterogeneity= 0.323) (**Figure 2**).

Findings from cross-sectional studies. Overall, combining effect sizes from 9 studies [38, 41, 40, 42, 43, 45-47, 49], that included 27296 participants and 3646 cases of depressive patients, revealed that high zinc status (a combination of dietary zinc and serum zinc concentration) was inversely associated with risk of depression (RR: 0.61; 95% CI: 0.51, 0.70) (**Figure 3),** with a low between-study heterogeneity (I^2 = 0.0 %, P = 0.420).

Study quality

Based on the Cochrane tools used, the seven RCTs presented high methodologic quality [19, 20, 52, 51, 53, 30, 31] (**Supplementary Table 2**). All cohort studies were recorded to have good quality [39, 44, 48] (**Supplementary Table 3**). In the cross-sectional studies, four studies were good [42, 43, 45, 41], however the remaining studies were scored as moderate to low quality [38, 46, 47, 40, 49] (**Supplementary Table 4**).

Discussion:

The present systematic review and meta-analysis of observational and RCTs studies sought to explicate the impact of zinc status and supplementation on depression development and treatment. First, our meta-analysis of cross-sectional studies revealed that inadequate zinc status (a combination of dietary zinc and serum zinc concentration) is prevalent among depressed patients. Second, we found that the highest level of zinc intake was associated with a 28% reduced risk of depression in the analysis of prospective cohort studies. Third, the result of the RCTs analysis confirmed the findings from observational studies regarding lower levels of zinc in depressed patients, and highlighted that zinc supplementation can significantly reduce depression score in depressive patients, when supplementing with zinc, in the absence of anti-depressant medications (monotherapy). Finally, this meta-analysis showed zinc supplementation had a beneficial effect on depression symptoms in patients with a depression score of 20-30 (mild to moderate depression).

Findings from the present updated meta-analysis are in line with the previous meta-analysis that shows an inverse association between zinc status and risk of depression [22, 21], the potential to alleviate depression symptoms following zinc supplementation, and, adding to previous studies, that only zinc monotherapy significantly affected depression score. Furthermore, based on the current meta-analysis, the largest antidepressant effect of zinc supplementation was reported in mild to moderately depressed subjects.

Micronutrient deficiencies are reported to be more frequent in depressed patients compared to healthy individuals [45, 55] . Indeed, deficiencies in key vitamins and minerals can interrupt the brain functioning and increase levels of stress. Zinc is one of the most important micronutrients, and plays a major role in the ability to regulate biological and psychological factors, and its inextricable association with depression has received much attention in field of nutrition and psychology in contemporary research. Zinc deficiency is known to increase Reactive Oxygen Species (ROS) and oxidative stress, and both are involved in the physiopathology of depression

[55, 56, 15]. Second, it is accepted that synaptic zinc is an N-methyl-D-aspartate (NMDA) receptor antagonist [57, 58]; indeed, NMDA antagonists were found to be therapeutically targeted in studies of depression treatment [55, 59]. Further, according to animal-based investigations, blockade of NMDA receptors can eliminate the beneficial effects of zinc on depressive related symptoms, suggesting that NMDA receptors are a mediator of the antidepressant properties of zinc [60]. Furthermore, the effect on hormonal regulation, cortisol, cellular immune response [61], neurogenesis, neural plasticity, and expression of hippocampal and cortical brain-derived neurotrophic factor (BDNF) [30, 55] are considered as additive explanations for the beneficial effect of zinc in patients with depression.

In the meta-regression models; age, dose of zinc supplementation, study duration, and baseline depression score made significant contributions to the change of depression. Indeed, higher doses of supplementation, longer intervention duration , and older adults with severe depression, respectively, were associated with an enhanced effect of zinc supplementation for decreasing depression score. However, due to the limited available evidence, these findings should be interpreted with caution

This meta-analysis study included several lines of evidence; cross-sectional, prospective cohort, and intervention studies; which strongly support the zinc-depression relationship. While original [50, 19, 20] intervention studies have reported zinc may be efficacious as an adjunctive therapy for depression, our study revealed that mood-enhancing properties of zinc supplementation can be observed in depressed patients who supplement with zinc in the absence of anti-depressant medications (zinc monotherapy). This is important, because, while prescribers continue antidepressant therapy, early antidepressant discontinuation is widespread in the community of depressed patients [62]; in addition, relapses are prevalent and costly to healthcare systems [62, 63].

A strength of the present meta-analysis is that it was the first to examine the effect of zinc monotherapy compared to placebo in depressed patients in RCTs. In addition, the dose–response relationship between zinc supplementation on depression status was in investigated, albeit a limited number of studies were eligible for analysis. However, in addition to the strengths and novelty of the present meta-analysis, there are some limitations that should be considered. Regarding the cohort studies, most included cohort studies in this meta-analysis had adjusted for potential confounders, including age, gender, socioeconomic status, BMI, physical activity, smoking status, but only two studies had adjusted for other micronutrients, including vitamin B family and vitamin D intake, as a confounder of risk of depression development [43, 47]. The different confounder adjustments among studies may lead to bias of the pooled results. Dietary zinc intake has been the main method for zinc status evaluation in observational studies, which is prone to measurement errors in zinc status assessment. Inconsistent criteria were used to diagnose depression among the observational studies included in the meta-analysis, which conceivably could have affected the strength of the association between zinc status and depression status.

Regarding RCTs, we are not aware of an intervention study to have examined the effect of zinc supplementation on healthy subjects, and our results may, therefore, not be generalizable to a healthy population. Furthermore, in the present analysis, the number of eligible studies was small; however, comparatively, the previous meta-analysis in this field was conducted in fewer trials. The Beck depression scale used to determine depression status in all included studies; however, various thresholds in Beck scale criteria to categorize depression were applied in the RCTs; hence, it was difficult to distinguish the true effect of zinc supplementation on the severity of depression. All of the eligible RCTs followed their participants for less than six months, therefore, whether the effect of zinc supplementation persists for a longer period of time remains unknown, and therein represents a viable avenue for further research. An important limitation present in most of

the included studies was that they were conducted in a relatively low range of geographical locations (3 from 4 cohort studies established in Australia [39, 48], and 6 from 8 RCTs conducted in Iran[20, 52, 51, 30, 31]), thus, the association between zinc status and risk of depression may not be congruent in different populations, and clearly warrants further investigation. Although we conducted several subgroup analyses, we were unable to adequately detect the sources of heterogeneity.

In conclusion, our findings advocate the preventative and therapeutic effect of zinc, compared to traditional antidepressant therapy, as a cost-efficient, efficacious, alternative approach. Future studies are needed to elucidate the effects of zinc intake on the depression-related symptoms in healthy individuals and in wider geographical locations.

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Table 1- Characteristics of observational studies evaluating the zinc-depression association

Table 2- Characteristics of randomized controlled trials evaluating the effect of zinc supplementation on depression status.

Table 3- Meta-analysis showing the effect of zinc supplementation on depression score based on several subgroups; all analyses were conducted using the random-effects model

Table 4- Effect of zinc supplementation on weighted mean difference controlling for age, baseline depression score, dose of zinc supplementation and the study duration (conducted for the random-effects model

Table 1- Characteristics of observational studies evaluating the zinc-depression association

Author (Year)	Study name (Location)	Study design (follow-up year)	Number of participants (Age)	Depressive patients (N)	Zinc measurement	Depression scale	RR (95 % CI)	Factors adjusted for in analyses (Multivariable)
Jacka (2012) [42]	GOS[1] (Australia)	Cross-sectional	1023 (49-60)	60	Dietary zinc intake	SCID-I/NP[2]	0.24 (0.08, 0.76)	Age, socioeconomic factors, physical activity, alcohol consumption, smoking, energy intake, BMI and supplementation
Jung (2017) [43]	BASE-II[3] (Germany)	Cross-sectional	1514 (<18)	238	Plasma Zinc	CES-D[4]	0.67 (0.46, 0.97)	Sex, age, and body mass index, hypothyroidism, serum vitamin D3and vitamin B12, plasma CRP, cognitive impairment, poor sleep quality, Morbidity index.
Lehto (2013) [44]	KIHDRIS[5] (Finland)	Prospective cohort (20 years)	2317 (54.3)	60	Dietary zinc intake	HPL[6]	0.94 (0.52, 1.69)	Age, baseline depression severity, smoking, alcohol use, physical exercise and the use of dietary supplements
Li (2018) [45]	NHANES[7] (USA)	Cross-sectional	14834 (18<)	1367	Dietary zinc intake	PHQ-9[8]	0.70 (0.47, 1.04)	Age, gender, BMI, race, educational level, smoking status, family income, work activity, recreational activity, hypertension, diabetes, and total daily energy intake
Maserejian (2012) [46]	BACH[9] (USA)	Cross-sectional	3708 (50.5)	753	Dietary zinc intake	CES-D	0.72 (0.52, 0.93)	Age, race/ethnicity, socioeconomic status, BMI, physical activity, smoking status, total energy intake, any antidepressant/antipsychotic medication use, cardiac disease, and arthritis/rheumatism

Miki (2015) [47]	FNHS[10] (Japan)	Cross-sectional	2006 (41)	557	Dietary zinc intake	CES-D	0.63 (0.45, 0.87)	Age, sex, and site, marital status, job grade, shift work, physical activity, smoking, alcohol consumption, intake of folate and vitamin C and vitamin B6 and vitamin B12 and polyunsaturated fatty acids
Vashum (2014) [48]	HCS[11] (Australia)	Prospective cohort (5)	2092 (<18)	270	Dietary zinc intake	CES-D	0.73 (0.44, 1.19)	Education, household income, hypertension, BMI, and energy intake
	ALSWH[12] (Australia)	Prospective cohort (6)	9738 (<18)	1830			0.70 (0.55, 0.90)	
Yary (2012) [49]	No brand (Malayia)	Cross-sectional	402 (32.54)	122	Dietary zinc intake	CES-D	0.50 (0.30, 0.84)	Sex, age, BMI, monthly expenses, close friends, living on campus, smoking, physical inactivity, education, and marital status
Das (2020) [39]	CHAMP (Australia)	Prospective cohort (3)	1705 (≥70)	83	Dietary zinc intake	GDS	0.41 (0.20, 0.85)	Age, body mass index, marital status, living arrangement, income, meal service, smoking, alcohol intake, comorbidity and energy, antidepressant medication
Anbari (2020) [38]	No brand (Iran)	Cross-sectional	297 (64)	125	Serum/dietary zinc	GDS	1.26 (0.62, 2.34)	age, BMI, sex, CVD, marital status, smoking, status, education, rheumatoid arthritis, hypertension, diabetes, hyperlipidemia, digestive disease
Nakamura (2019) [40]	Eat-Well (Japan)	Cross-sectional	2089 (18-79)	144	Dietary zinc intake	Kessler's six item psychological distress scale (K6)	0.6 (0.32, 1.12)	age and sex, smoking, alcohol drinking, body mass index, shift work, and intake of Vitamin C, B6, B12, folic acid, and PUFA; medications for hypertension, hyperlipidemia, and diabetes

Nguyen (2019) [41]	Shika study (Japan)	Cross-sectional	1423 (≥65)	280	Dietary zinc intake	GDS	0.58 (0.38, 0.9)	age, BMI, living status, having a job status, married status, smoking status, alcohol consumption, total energy, hypertension, diabetes, hyperlipidemia

1 Geelong Osteoporosis Study; 2 Structured Clinical Interview for DSM-IV-TR Research Version Non-patient edition; 3 Berlin Aging Study II; 4 Center for Epidemiological Studies Depression scale; 5 Kuopio Ischemic Heart Disease Risk Factor Study; 6 Human Population Laboratory Depression Scale; 7 National Health and Nutrition Examination Survey; 8 Patient Health Questionnaire; 9 Boston Area Community Health Survey; 10 Furukawa Nutrition and Health Study; 11 Hunter Community Study; 12 Australian Longitudinal Study on Women's Health

Table 2- Characteristics of randomized controlled trials evaluating the effect of zinc supplementation on depression status.

The first author (year)	Country	No. of participants (Gender)	Design	Depression status	Study duration (week)	Depression scale	Zinc type (Dose mg/day)	Control group status	Results
Nazarinasab (2017) [50]	Iran	Int[1]: 29, Cont[2]: 29 (Both)	Parallel	Depressed patients	8	BDI II[3]	Zinc sulfate (25)	Anti-depressant drug	Significant effect on Beck score in in combination with selective serotonin inhibitors reduces depression
Nowak (2003) [19]	Poland	Int:6, Cont:8 (Both)	Parallel	Depressed patients	12	HDRS[4] and BDI II	Zinc (25)	Anti-depressant drug	Significant effect on Beck and HDS score in patients with major depression
Ranjbar, (2013) [20]	Iran	Int:21, Cont:17 (Both)	Parallel	Depressed patients	12	BDI II	Zinc sulfate (25)	Anti-depressant drug	Significant effect on Beck score together with the antidepressant drug in patients with major depression
Ranjbar, (2014) [52]	Iran	Int:21, Cont:17 (Both)	Parallel	Depressed patients	12	HDRS	Zinc sulfate (25)	Anti-depressant drug	Significant effect on HDRS score together with antidepressant drug in patients with major depression
Salari, (2015) [51]	Iran	Int:21, Cont:22 (Both)	Parallel	Depressed patients	12	BDI II	Zinc sulfate (220)	Placebo	Significant effect on Beck score in MS patients with major depression
Siwek (2009) [53]	Poland	Int:30, Cont:30 (Both)	Parallel	Depressed patients	12	HDRS, BDI II, CGI[5] and MADRS[6]	zinc hydro aspartate (25)	Anti-depressant drug	No significant effect in antidepressant treatment nonresistant patients, significant effect in antidepressant treatment resistant patients
Solati, (2015) [30]	Iran	Int:22, Cont:24 (Both)	Parallel	Both	12	BDI II	Zinc gluconate (30)	Placebo	Zinc monotherapy improves mood in overweight or obese subjects
Yosaee (2018) [31]	Iran	Int:30, Cont:30 (Both)	Parallel	Depressed patients	12	BDI II	Zinc gluconate (30)	Placebo	Significant effect on Beck score in depressed patients

1. intervention, 2. control, 3. Beck Depression Inventory, 4. Hamilton Depreaaion Rating Scale, 5. Clinical Global Impression, 6. Montgomery–Asberg Depression Rating Scale

Table 3- Meta-analysis showing the effect of zinc supplementation on depression score based on several subgroups; all analyses were conducted using the random-effects model

Study group	Number of studies	Meta-analysis		Heterogeneity			
		(95% CI)	*P* value	Q statistic	P within group	I^2(%)	*P* between group
Overall	7	-4.15 (-6.56, -1.75)	<0.001	30.11	<0.001	80.1	--
Depression status							
Depressed patients	6	-4.58 (-7.55, -1.60)	0.003	30.06	<0.001	83.4	0.816
Mixed population	1	-2.92 (-5.39, -0.44)	0.021	0.00	--	--	
Depression scale							
Beck	7	-4.16 (-6.56, -1.75)	<0.001	30.11	<0.001	80.1	0.949
Hamilton	3	-8.04 (-15.00, -1.07)	0.024	20.99	<0.001	90.5	
Duration							
Shorter period (≤ 12 weeks)	6	-4.73 (-8.25, -1.19)	0.009	29.99	<0.001	83.3	0.728
Longer period (> 12 weeks)	1	-3.32 (-4.40, -2.24)	<0.001	0.00	--	--	
zinc prescription							
Monotherapy	3	-5.05 (-7.55, -2.54)	<0.001	4.35	0.114	54	0.024
Adjunctive therapy	4	-3.70 (-7.80, 0.39)	0.076	20.66	<0.001	85.5	
Control group status							
Without antidepressant	3	-5.05 (-7.55, -2.54)	<0.001	4.35	0.114	54	0.024
With antidepressants	4	-3.70 (-7.80, 0.39)	0.076	20.66	<0.001	85.5	

Table 4- Effect of zinc supplementation on weighted mean difference controlling for age, baseline depression score, dose of zinc supplementation and the study duration (conducted for the random-effects model)

Variable	Estimated coefficient	Standard error	I-squared residual	P- Value
Age	-0.134	0.037	83.50	0.005
Zinc supplementation dose (mg/d)	-0.178	0.049	80.95	0.006
Duration (weeks)	-0.321	0.116	85..28	0.015
Baseline depression score	-0.195	0.054	85.76	0.006

Legend of Figures

Figure 1- Effect of zinc supplementation on depression status in randomized controlled trials

Figure 2- Relative risk (RR) of depression status for the highest versus lowest category of zinc intake among prospective cohort studies.

Figure 3- Relative risk (RR) of depression status for the highest versus lowest category of zinc status among cross-sectional studies.

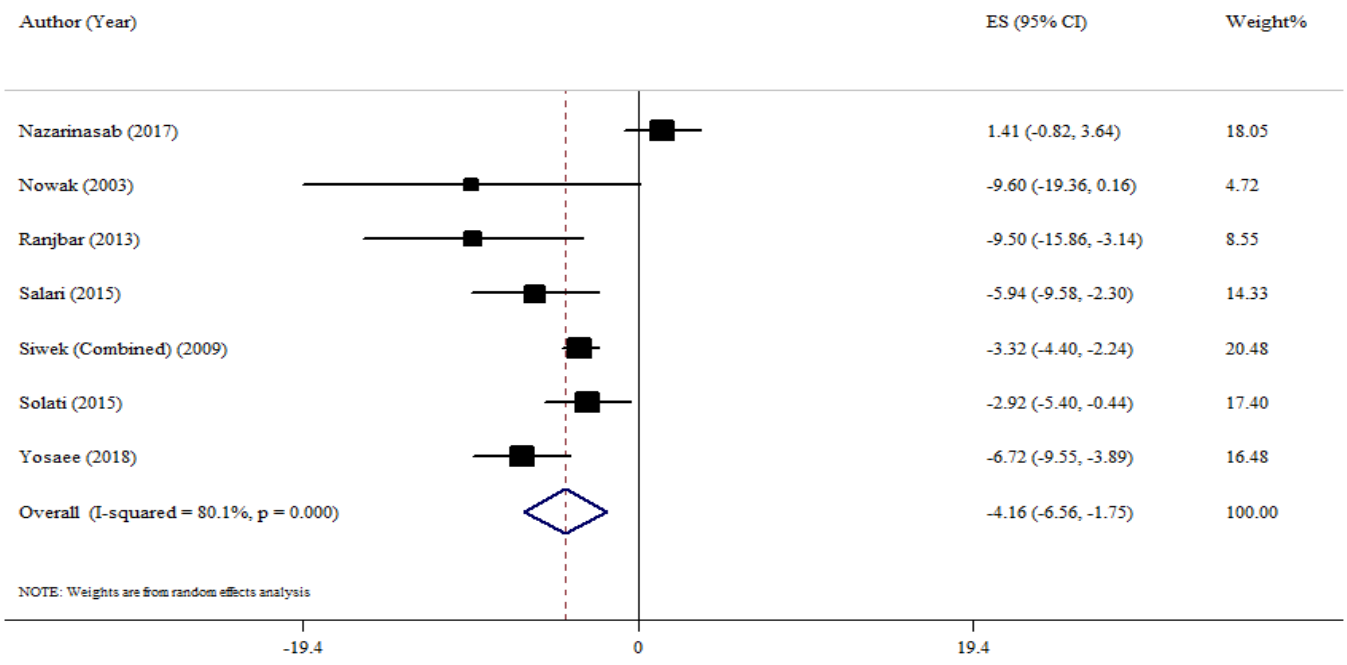

Figure 1- Effect of zinc supplementation on depression status in randomized controlled trials

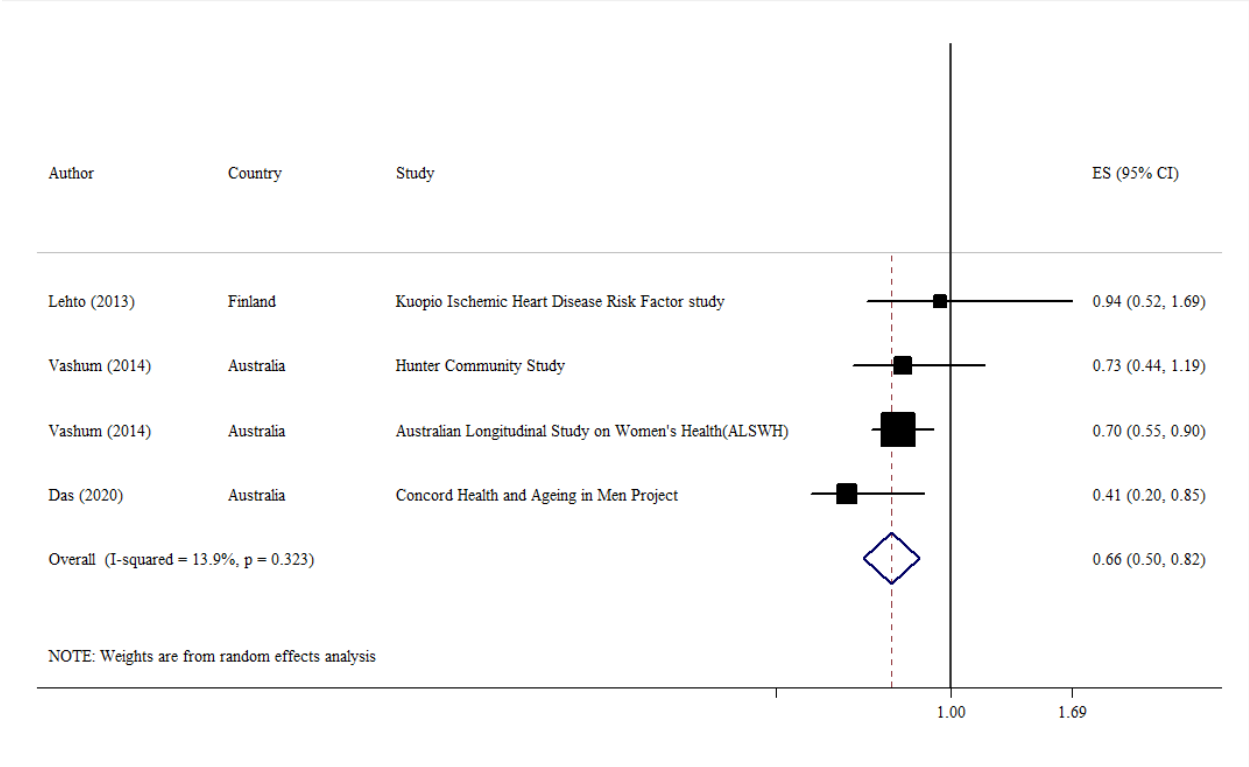

Figure 2- Relative risk (RR) of depression status for the highest versus lowest category of zinc intake among prospective cohort studies.

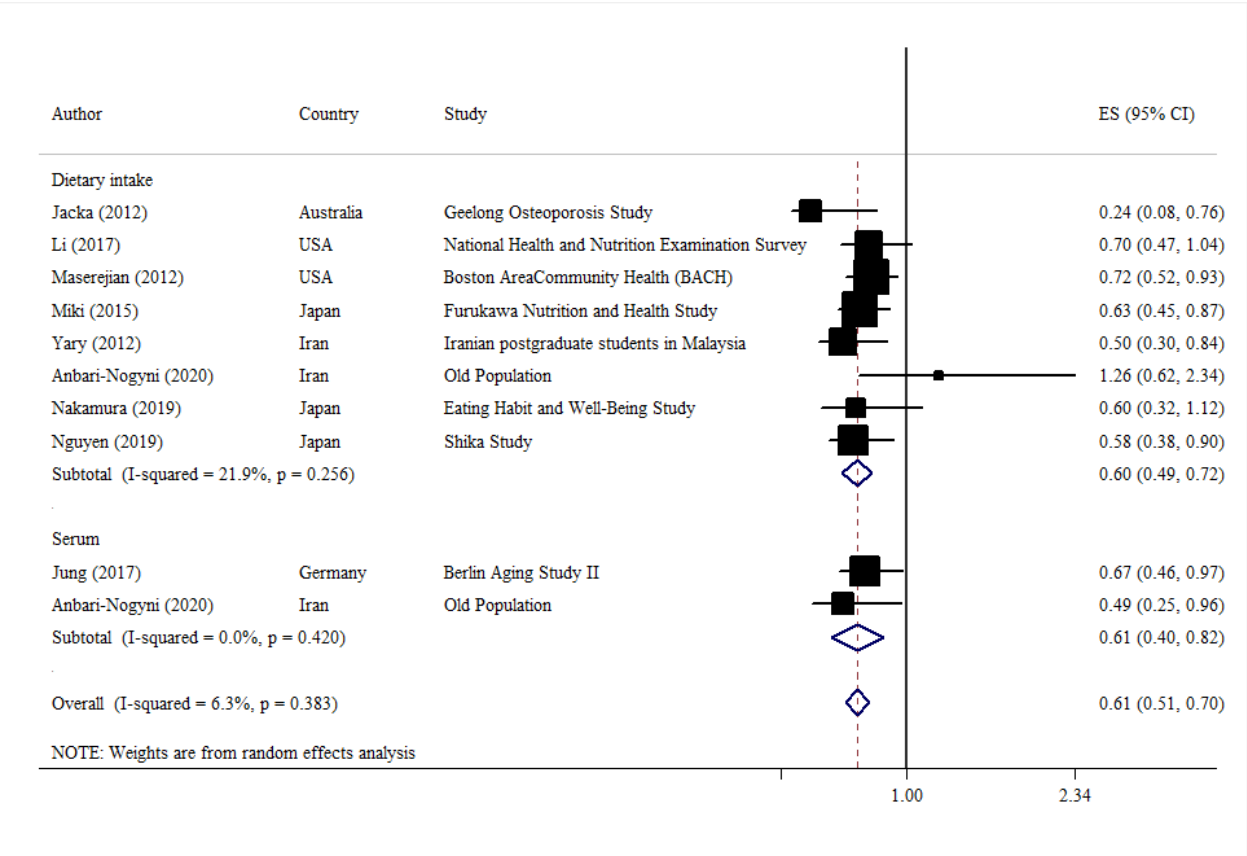

Figure 3- Relative risk (RR) of depression status for the highest versus lowest category of zinc status among cross-sectional studies.