

DR. NICOLA ANNE GRAY (Orcid ID : 0000-0001-7024-3903)

Article type : Review Article

Title: Zinc and atopic dermatitis: a systematic review and meta-analysis

Authors:

N.A. Gray,^{*1,2} A. Dhana,^{*1} D.J. Stein,^{2,3} N.P. Khumalo.^{1,4}

*Both authors contributed equally to this work.

¹Division of Dermatology, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa

²Department of Psychiatry and Mental Health, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa

³SA MRC Unit on Risk and Resilience in Mental Disorders

⁴Hair and Skin Research Laboratory, Division of Dermatology, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa

Corresponding author:

Nicola A. Gray

Division of Dermatology, Groote Schuur Hospital, Observatory, Cape Town, 7935

Tel: 021-404-5275

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jdv.15524

This article is protected by copyright. All rights reserved.

E-mail: nicolagrayemail@gmail.com

Funding:

N.A. Gray is funded by the Discovery Foundation.

Conflict(s) of interest:

None.

Word count:

Abstract: 300 (of max 300)

Body: 2924 (of max 3000)

ABSTRACT

Zinc plays a central role in skin integrity via barrier and immune mechanisms and may also be relevant in the pathogenesis of atopic dermatitis (AD). However, little is known about the relationship between zinc and AD. We performed a systematic review to determine 1) the association between zinc levels or zinc deficiency and AD and 2) the efficacy of oral zinc supplementation in the treatment of AD. We searched PubMed, Scopus, Web of Science and article references for observational studies on zinc levels or zinc deficiency in participants with AD versus controls and for RCTs on zinc supplementation in AD. For observational studies, we calculated pooled standardised mean differences (SMDs) or odds ratios (ORs) along with 95% confidence intervals (CIs) using a random effects model. We included 14 observational studies and 2 RCTs. The pooled SMD demonstrated

significantly lower serum (SMD 0.66, 95% CI 0.21-1.10, $p=0.004$), hair (SMD 0.95, 95% CI 0.38-1.52, $p=0.001$) and erythrocyte (SMD 0.95, 95% CI 0.38-1.52, $p=0.001$) zinc levels in participants with AD compared to controls. Pooled unadjusted data from 3 studies showed a non-significant increased odds of AD in those with zinc deficiency compared with those without zinc deficiency (OR=1.50, 95% CI 0.71-3.16, $p=0.28$). One RCT of oral zinc supplementation among AD patients with zinc deficiency showed improvement in extent and severity of AD, while another RCT among all AD patients showed no significant improvement. All the studies were of low or moderate quality. We conclude that low serum, hair, and erythrocyte zinc levels are associated with AD. However, the poor quality of included studies make interpretation of these results problematic. High quality observational studies are needed to confirm the association between low zinc levels and AD, and RCTs are required to evaluate the merit of zinc supplementation for the treatment or prevention of AD.

Key words: zinc, micronutrients, trace elements, atopic dermatitis, eczema

BACKGROUND

Atopic dermatitis (AD) is a chronic, inflammatory, pruritic skin disease with a global prevalence ranging from 0.9 to 22.5%. It affects developing as well as developed countries,¹ and is associated with significant morbidity, including a significantly higher prevalence of sleep and mood disorders.² Reduction in quality of life is associated with intractable pruritis and is proportional to disease severity.^{3,4} Atopic dermatitis in infancy is also an independent risk factor for the development of other allergic diseases later in life, including food allergy, asthma, and allergic rhinitis.⁵

Zinc is an essential micro-nutrient that catalyses enzyme activity, contributes to protein structure, and regulates gene expression.⁶ It is also essential for the functioning of the innate and adaptive immune systems.^{7,8} Five percent of total body zinc is found in the skin, with epidermal concentrations highest

in the stratum spinosum.⁹ In fact, zinc has been shown to promote normal keratinocyte differentiation and reduce pro-inflammatory actions of keratinocytes.⁹ AD has a complex pathogenesis with barrier defects ('outside-in' hypothesis), immunologic alterations ('inside-out' hypothesis), and an altered microbiome with increased *s.aureus* colonisation playing a role.¹⁰⁻¹² Although the exact nature of the relationship between zinc status and AD remains poorly understood, it is hypothesized that low zinc concentrations may contribute to AD via all 3 mechanisms. First, zinc is a component of matrix metalloproteinases that are involved in epidermal remodelling, whereas zinc finger motifs play a role in expression of filaggrin, a key protein required for formation of the skin barrier.¹³ Second, application of topical zinc oxide particles in mechanically damaged mice skin has been shown to decrease pro-inflammatory cytokines such as IL-1 and IL-6 and increase anti-inflammatory cytokines such as IL-10.¹³ Murine models have also shown that a zinc deficient diet results in several features of AD including increased skin dryness, itching, and transepidermal water loss, as well as elevated serum IgE, skin *s.aureus*, and mast and eosinophil cell counts on histology.¹⁴ Third, although the relationship between endogenous zinc status in humans and *s.aureus* colonisation remains unknown, zinc oxide particles have in vitro antimicrobial activity against *s.aureus*.¹⁶

A regular dietary intake of zinc is needed as there is no storage form of zinc in the body, and children from low- middle- income countries are still vulnerable to zinc deficiency.^{17, 18} Despite the important role of zinc in the skin, little is known about the relationship between zinc deficiency and AD, and existing studies show conflicting results. This is in stark contrast to research regarding the role of zinc in intestinal epithelial disease.¹⁹ As a first step in addressing this gap in the literature, we performed a systematic review and meta-analysis to determine the relationship between zinc and AD.

METHODS

This systematic review was conducted using a prospective protocol based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.²⁰ Details of the protocol were registered on Prospero (CRD42018093560). Two authors (NG and AD) were independently involved in study selection, data extraction, and quality assessment, and disagreements were resolved by discussion with a third author (NK) if consensus could not be reached.

Literature search strategy

We searched PubMed, Scopus and Web of Science on 4th December 2017. The search strategy for each database is detailed in supplementary table 1. The reference lists of included articles were also reviewed for additional studies.

Eligibility Criteria

We included any peer-reviewed English language article that examined the relationship between zinc and AD and used a case-control, cross-sectional, cohort, or randomised control trial (RCT) study design. Studies that enrolled both children and/or adults were included. Any criteria for AD diagnosis was accepted. Included observational studies had to measure zinc levels and report mean differences or relative risks (RRs) [odds ratios (ORs) or hazard ratios (HRs)] with 95% confidence intervals (CIs), or have sufficient data to compute them. Studies were excluded if they did not contain objective data on zinc levels or used dietary assessments of zinc intake only. RCTs were included if there was a zinc intervention arm.

Study selection and data abstraction

Titles and abstracts identified by the search were screened, and then full texts of selected articles were reviewed. We extracted information from each study using a pre-defined data extraction form designed for this review.

Study quality assessments

The Newcastle-Ottawa Scale was used to assess the quality of nonrandomised studies in the review.²¹

²² The Cochrane Risk of Bias Tool was used to assess quality of RCTs.²³

Statistical analysis

We conducted meta-analyses using Stata, version 13, (StataCorp LP, College Station, TX). There were sufficient data to perform four meta-analyses. The first three compared mean concentrations of serum, hair, and erythrocyte zinc between participants with AD and those without AD. Standardized mean difference (SMD) was chosen as the summary statistic rather than weighted mean difference because studies measured zinc levels on difference scales. The final meta-analysis examined the association between zinc deficiency and AD. Since no RR estimates were provided, crude ORs along with 95% CIs were calculated from 3 included studies. For all analyses, we chose a random effects model using the DerSimonian-Laird method²⁴. To assess the degree of heterogeneity across studies, we used the I^2 statistic and P-value of χ^2 squared test. An I^2 value of 25%, 50%, and 75% was considered low, moderate, and high heterogeneity, respectively.²⁵

The following additional tests were performed if 5 or more studies were present. Subgroup analysis using univariate meta-regression was performed to try and explain potential between-study heterogeneity. The following subgroups were chosen *a priori*: study quality (<6 vs ≥ 6 stars); region (Europe vs other); age and/or sex matched controls (yes vs no); age (children only vs other); total

study size ($n < 100$ vs $n \geq 100$) and type of control group (healthy vs other). As a sensitivity analysis, a meta-influence plot was used to analyse the influence of individual studies on the overall effect size. We used a funnel plot and Egger's linear regression test to assess publication bias. All p values were two sided and alpha was set at 0.05.

RESULTS

Study selection and characteristics

Results of the study selection process and reasons for exclusion are summarised in Fig. 1. Fifteen studies were included in the review: 11 case-control²⁶⁻³⁶, 1 cross-sectional³⁷, 1 cohort³⁸, and 1 RCT,³⁹ as well as 1 study that combined a case-control study with a subsequent RCT.⁴⁰

The main characteristics of the 14 observational studies included in the review are summarised in Table 1. The two RCTs are discussed in a separate section below. Three studies were from the United Kingdom; 2 each from Hong Kong, South Korea, Turkey, and Japan; and one each from Bangladesh, Egypt, and Italy. Nine studies included only children,^{27-30, 32, 33, 35, 38, 40} 3 included only adults,^{31, 36, 37} and 2 included children and adults.^{26, 34} Only three studies included participants over the age of 35 years,^{31, 36, 37} and only 2 studies focused on children aged under 5 years.^{27, 35} The proportion of male cases ranged from 35.6% to 66.7%, and male controls ranged from 49.0% to 65.9%. Eight of the 15 included observational studies and RCTs used pre-defined criteria for AD: 5 used Hanifin and Rajka's criteria,^{32-34, 39, 40} 2 used the Japanese Dermatologic Society Diagnostic criteria,^{37, 38} and one used consensus conference criteria.³⁵ No specific criteria were used in the remaining 7 studies with 1 study relying on self-report.³⁷ Two studies also took IgE measurements into consideration.^{29, 30}

Method of zinc determination

Ten studies determined zinc concentration from serum,^{26-30, 32-34, 36, 37} 3 from hair,^{30, 38, 40} 2 from erythrocytes,^{34, 35} and 1 from leukocytes and plasma³¹. In terms of instruments, 7 studies used atomic absorption spectrophotometry (AAS),^{26-28, 30, 31, 34, 36} 4 used inductively-coupled mass spectrophotometry (ICP-MS),^{32, 33, 37, 40} 2 used proton-induced x-ray emission (PIXE),^{29, 38} and 1 used mass spectrophotometry but did not specify the type.³⁵

Study quality assessment

Study quality assessment scores for observational studies are shown in Supplementary Table 2. All studies were judged to be of low to moderate quality. For participant selection, none of the 12 case control studies scored more than 2 out of 4 stars. None described the representativeness of cases, 4 used hospital-based controls,^{27, 28, 32, 33} and 8 provided minimal or no description of where the controls were sourced from.^{26, 29-31, 34-36, 40} For comparability of groups, 5 studies did not match or adjust for any potential confounders,^{28, 32, 33, 36, 37} and 3 studies matched or adjusted for only 1 factor.^{26, 27, 38} For ascertainment of exposure, all studies used an objective measure of zinc determination and the same method was used for cases and controls, but no studies provided non-response rates or described non-respondents. For assessment of outcome, the cross-sectional study relied on self-report of AD,³⁷ whereas the cohort study used independent blind assessment but only followed participants up to 10 months of age.³⁸

Supplementary Table 3 shows the risk of bias assessments for the 2 RCTs. None of the trials reported adequate random sequence generation or allocation concealment. Both trials reported adequate blinding of personnel and outcome assessors, although 1 trial did not report adequate blinding of participants. Finally, follow-up was adequate in one trial but unclear in the other.

Cross sectional and case control studies

Eight case-control studies including 840 participants assessed mean serum zinc levels (Supplementary Table 4). Overall, serum zinc levels were significantly lower in participants with AD than in controls (SMD 0.66, 95% CI 0.21-1.10, $p=0.004$) (Fig. 2). Heterogeneity was high ($p<0.00$, $I^2=87.7\%$).

Univariate meta-regression did not reveal significant heterogeneity by study characteristics (Supplementary Table 5). There was some evidence of funnel plot asymmetry, although Egger's test was non-significant ($p=0.47$). In influence analysis, the pooled estimate remained significant and ranged from 0.43 (95% CI, 0.27-0.60) to 0.73 (95% CI, 0.57-0.90). One study assessed median serum zinc levels, finding no significant difference between participants with AD and controls (cases: median 11.5 $\mu\text{mol/L}$; controls: median=11.5 $\mu\text{mol/L}$, $p=0.29$).²⁸ Three case-control studies including 97 participants assessed mean hair zinc levels (Supplementary Table 4). Overall, hair zinc levels were significantly lower in participants with AD than in controls (SMD 0.95, 95% CI 0.38-1.52, $p=0.001$) (Fig. 3). Heterogeneity was high ($p=0.06$, $I^2=64.5\%$). Two case-control studies including 159 participants assessed mean erythrocyte zinc levels. (Supplementary Table 4). Overall, serum zinc levels were significantly lower in participants with AD than in controls (SMD 1.21, 95% CI 0.90-1.53, $p<0.001$) (Fig. 4). Heterogeneity was moderate ($p=0.23$, $I^2=30.6\%$). One study measured zinc concentrations in both plasma and leukocytes, but results were not significant ($p>0.05$).³¹

Six studies analysed zinc levels by AD severity.^{27, 28, 31, 33-35} Two studies used the SCORing Atopic Dermatitis (SCORAD) index only,^{34, 35} 1 used SCORAD, Nottingham Eczema Severity Score (NESS) AND Children's Dermatology Life Quality Index (CDLQI),³³ and the remaining 3 studies used non-validated scoring systems. Four studies found no difference in zinc levels with increasing AD severity.^{27, 28, 31, 35} One study found that serum zinc levels were inversely related to long term disease severity as measured by NESS ($r=-0.24$, $p<0.05$), but found no correlation with SCORAD or CDLQI.³³ Another study found that erythrocyte zinc levels were inversely related to SCORAD ($r=-0.791$, $p<0.001$), but not serum zinc.³⁴

Zinc and incident AD

A prospective mother child birth cohort assessed the association between infant or maternal hair mineral content at age 1 month and AD diagnosis at age 10 months after adjusting for family history of AD.³⁸ Neither infant nor maternal hair zinc at age 1 month was related to AD status at 10 months ($p>0.2$).

Zinc deficiency and AD

Pooled unadjusted data from 3 studies of 2119 participants showed a non-significant increased odds of AD in those with zinc deficiency compared with those without zinc deficiency (RR=1.50, 95% CI 0.71-3.16, $p=0.28$). Heterogeneity was high (χ^2 - squared $p=0.039$; I-squared 69.3%).

RCTs of zinc supplementation in AD

Two RCTs of zinc supplementation in AD were identified but reported different outcome measures, precluding meta-analysis.^{39, 40} Ewing et al. published a randomised double-blind placebo controlled trial of oral zinc sulphate supplementation in children with AD.³⁹ There was no significant difference between groups for several outcomes: weight of steroid or emollient used, surface area score, erythema score, or combined disease severity score. However, itch scores at 8 weeks were significantly higher in children receiving zinc supplementation ($p=0.01$). Kim et al. randomized children with AD and low hair zinc levels to receive oral zinc oxide supplementation or no zinc supplementation.⁴⁰ Blinded independent dermatologists scored AD severity but participants were not blinded, and the control group did not receive a placebo. The zinc supplementation group had a significant increase in hair zinc levels, as well as significant improvement in extent and severity of AD ($p=0.044$), pruritus ($p<0.001$), and transepidermal water loss ($p=0.015$) compared with the group not receiving supplementation. However, differences in sleep scores were not significant ($p>0.05$).

DISCUSSION

In this systematic review and meta-analysis of zinc and atopic dermatitis, we found significantly lower mean serum, hair, and erythrocyte zinc levels in participants with eczema compared with controls. Using unadjusted data, there was also a non-significantly increased odds of AD in those with zinc deficiency compared with those without zinc deficiency. Finally, two RCTs of zinc supplementation among AD patients revealed significant improvements in extent and severity of disease only in patients with low zinc levels but not in all patients. However, the lack of high quality studies (both observational and interventional), and the high degree of between-study heterogeneity, makes interpretation of these results difficult.

It is estimated that Africa has the highest rates of zinc deficiency globally, with pregnant women and children under 5 years most at risk globally.⁴¹ It is therefore notable that none of the studies identified for inclusion in this review were conducted in sub-Saharan Africa, and even among the studies from low- and middle-income countries there was a relative lack of data on children under 5 years of age. These under-represented groups are even more relevant considering the rising prevalence of AD in developing countries, and the high relative burden of AD in younger children.^{1, 42, 43} Therefore, observational studies and randomised trials in Africa are warranted.

The lower mean zinc levels in AD patients compared with controls were consistent across all three substrates: serum, hair, and erythrocytes. However, as there is no storage form of zinc in the body, serum and erythrocyte zinc measurements reflect current, rather than medium- or long-term average zinc concentration. While dietary zinc intake (which was not included in this review) may provide helpful information on zinc status, it does not take into account zinc loss, which can occur via the skin, urine, or stool.⁴⁴ As hair grows at an average of 1cm per month, hair analysis may be a better indicator of average zinc levels rather than serum analysis which may be affected by acute fluctuations.^{45, 46} Interestingly, hair zinc has been shown to be a marker of marginal zinc deficiency

(MZD), as it has been used to detect subtle, asymptomatic zinc deficiency, which is reversible with zinc supplementation and which could not be detected using serum measurements.⁴⁵ It is hypothesized that the slow uptake of zinc by hair is reduced in states of decreased zinc availability.⁴⁵ Indeed, all of the individual studies using hair as a substrate for zinc analysis showed statistically significantly lower zinc concentrations in cases with AD compared with controls, whereas individual studies using serum zinc for analysis were not all significant.

The relationship between zinc levels and other atopic diseases is also still unclear. Zinc deficiency may exacerbate airway inflammation in asthma.⁴⁷ Some studies show an association between low zinc levels and asthma,⁴⁸⁻⁵² while others contradict these findings,⁵³⁻⁵⁶ and systematic review evidence is lacking. One study found no relationship between allergic rhinitis and plasma zinc and another study found no relationship between allergic rhinitis and dietary zinc intake.^{57,58}

We performed a comprehensive review of zinc and atopic dermatitis with meta-analyses for several different zinc substrates (serum, hair and erythrocytes). The concordant and significant results were further interrogated by looking for small study bias, performing influence analysis, and subgroup analysis. However, there are also several limitations to this review. First, we only searched the English literature and did not consider articles in non-peer-reviewed journals, thereby potentially omitting relevant studies. Second, since the pooled analysis for the association between zinc deficiency and atopic dermatitis used unadjusted data calculated from three studies, confounding likely biased the observed association. Third, studies were heterogenous in several aspects, such as type of controls, method of zinc determination, diagnostic criteria for AD, and geographic location. However, we used a random-effects model in our analyses to account for this variation, and most summary estimates were qualitatively similar. Nevertheless, caution should be exercised when interpreting the summary estimates. Finally, all studies were of low or moderate quality.

CONCLUSIONS

Our findings suggest that AD is associated with significantly lower serum, hair, and erythrocyte zinc levels. Additionally, there was a non-significant increased odds of AD among those with zinc deficiency compared with those without zinc deficiency, although the sample size for this comparison was small and only unadjusted data were available from individual studies. One RCT of oral zinc supplementation showed improvement in extent and severity of AD among AD patients with zinc deficiency, while another RCT showed no significant improvement among all AD patients. However, the low quality of included studies and high degree of heterogeneity mean that our findings must therefore be interpreted with caution, and, as such, the true relationship between zinc status and AD remains uncertain. High quality observational studies are needed to confirm the association between low zinc levels and AD, and RCTs are required to evaluate the merit of zinc supplementation for the treatment or prevention of AD.

ACKNOWLEDGEMENTS

N.A. Gray is funded by the Discovery Foundation.

REFERENCES

1. Odhiambo JA, Williams HC, Clayton TO, Robertson CF, Asher MI. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. *The Journal of allergy and clinical immunology*. 2009;124(6):1251-8.e23.
2. Eckert L, Gupta S, Amand C, Gadkari A, Mahajan P, Gelfand JM. Impact of atopic dermatitis on health-related quality of life and productivity in adults in the United States: An analysis using the National Health and Wellness Survey. *J Am Acad Dermatol*. 2017;77(2):274-9.e3.
3. Holm JG, Agner T, Clausen ML, Thomsen SF. Quality of life and disease severity in patients with atopic dermatitis. *Journal of the European Academy of Dermatology and Venereology : JEADV*. 2016;30(10):1760-7.
4. Kido-Nakahara M, Furue M, Ulzii D, Nakahara T. Itch in Atopic Dermatitis. *Immunology and allergy clinics of North America*. 2017;37(1):113-22.
5. Goksor E, Loid P, Alm B, Aberg N, Wennergren G. The allergic march comprises the coexistence of related patterns of allergic disease not just the progressive development of one disease. *Acta paediatrica (Oslo, Norway : 1992)*. 2016;105(12):1472-9.
6. Han TH, Lee J, Kim YJ. Hair Zinc Level Analysis and Correlative Micronutrients in Children Presenting with Malnutrition and Poor Growth. *Pediatric gastroenterology, hepatology & nutrition*. 2016;19(4):259-68.
7. Maggini S, Wintergerst ES, Beveridge S, Hornig DH. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. *The British journal of nutrition*. 2007;98 Suppl 1:S29-35.
8. Maares M, Haase H. Zinc and immunity: An essential interrelation. *Archives of biochemistry and biophysics*. 2016;611:58-65.
9. Ogawa Y, Kinoshita M, Shimada S, Kawamura T. Zinc and Skin Disorders. *Nutrients*. 2018;10(2).
10. Wang D, Beck LA. Immunologic Targets in Atopic Dermatitis and Emerging Therapies: An Update. *American journal of clinical dermatology*. 2016;17(5):425-43.
11. de Wit J, Totte JEE, van Buchem FJM, Pasmans S. The prevalence of antibody responses against *Staphylococcus aureus* antigens in patients with atopic dermatitis: a systematic review and meta-analysis. *The British journal of dermatology*. 2018;178(6):1263-71.
12. Geoghegan JA, Irvine AD, Foster TJ. *Staphylococcus aureus* and Atopic Dermatitis: A Complex and Evolving Relationship. *Trends in microbiology*. 2018;26(6):484-97.
13. Maarouf M, Vaughn AR, Shi VY. Topical micronutrients in atopic dermatitis-An evidence-based review. *Dermatol Ther*. 2018;31(5):e12659.
14. Makiura M, Akamatsu H, Akita H, Yagami A, Shimizu Y, Eiro H, et al. Atopic dermatitis-like symptoms in HR-1 hairless mice fed a diet low in magnesium and zinc. *Journal of International Medical Research*. 2004;32(4):392-9.
15. Takahashi H, Nakazawa M, Takahashi K, Aihara M, Minami M, Hirasawa T, et al. Effects of zinc deficient diet on development of atopic dermatitis-like eruptions in DS-Nh mice. *Journal of Dermatological Science*. 2008;50(1):31-9.
16. Kadiyala U, Turali-Emre ES, Bahng JH, Kotov NA, VanEpps JS. Unexpected insights into antibacterial activity of zinc oxide nanoparticles against methicillin resistant *Staphylococcus aureus* (MRSA). *Nanoscale*. 2018;10(10):4927-39.
17. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet*. 2013;382(9890):427-51.
18. Lowe NM, Fekete K, Decsi T. Methods of assessment of zinc status in humans: a systematic review. *The American journal of clinical nutrition*. 2009;89(6):2040s-51s.
19. Lazzerini M, Wanzira H. Oral zinc for treating diarrhoea in children. *Cochrane Database Syst Rev*. 2016;12:CD005436.

20. Moher D, Shamseer L, Clarke M, Gherzi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews*. 2015;4:1.
21. Wells G.A. SB, O'Connell D., Peterson J., Welch V., Losos M., Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analysis: Ottawa Hospital Research Institute; 2014 [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
22. Herzog R, Alvarez-Pasquin MJ, Diaz C, Del Barrio JL, Estrada JM, Gil A. Are healthcare workers' intentions to vaccinate related to their knowledge, beliefs and attitudes? A systematic review. *BMC public health*. 2013;13:154.
23. Higgins JPT GSe. *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0: The Cochrane Collaboration.; 2011 [updated March 2011. Available from: www.handbook.cochrane.org.
24. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177-88.
25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ (Clinical research ed)*. 2003;327(7414):557-60.
26. Amin MN, Liza KF, Sarwar MS, Ahmed J, Adnan MT, Chowdhury MI, et al. Effect of lipid peroxidation, antioxidants, macro minerals and trace elements on eczema. *Archives of Dermatological Research*. 2015;307(7):617-23.
27. David TJ, Wells FE, Sharpe TC, Gibbs AC. Low serum zinc in children with atopic eczema. *The British journal of dermatology*. 1984;111(5):597-601.
28. David TJ, Wells FE, Sharpe TC, Gibbs AC, Devlin J. Serum levels of trace metals in children with atopic eczema. *The British journal of dermatology*. 1990;122(4):485-9.
29. Di Toro R, Galdo Capotorti G, Gialanella G, Miraglia del Giudice M, Moro R, Perrone L. Zinc and copper status of allergic children. *Acta paediatrica Scandinavica*. 1987;76(4):612-7.
30. el-Kholy MS, Gas Allah MA, el-Shimi S, el-Baz F, el-Tayeb H, Abdel-Hamid MS. Zinc and copper status in children with bronchial asthma and atopic dermatitis. *The Journal of the Egyptian Public Health Association*. 1990;65(5-6):657-68.
31. Hinks LJ, Young S, Clayton B. Trace element status in eczema and psoriasis. *Clinical and Experimental Dermatology*. 1987;12(2):93-7.
32. Hon KL, Lui HK, Wang SS, Lam HS, Leung TF. Fish Consumption, Fish Atopy and Related Heavy Metals in Childhood Eczema. *Iranian Journal of Allergy Asthma and Immunology*. 2012;11(3):230-5.
33. Hon KL, Wang SS, Hung EC, Lam HS, Lui HH, Chow CM, et al. Serum levels of heavy metals in childhood eczema and skin diseases: friends or foes. *Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology*. 2010;21(5):831-6.
34. Karabacak E, Aydin E, Kutlu A, Ozcan O, Muftuoglu T, Gunes A, et al. Erythrocyte zinc level in patients with atopic dermatitis and its relation to SCORAD index. *Postepy Dermatologii i Alergologii*. 2016;33(5):349-52.
35. Toyran M, Kaymak M, Vezir E, Harmanci K, Kaya A, Ginis T, et al. Trace Element Levels in Children With Atopic Dermatitis. *Journal of Investigational Allergology and Clinical Immunology*. 2012;22(5):341-4.
36. Tasaki M, Hanada K, Hashimoto I. Analyses of serum copper and zinc levels and copper/zinc ratios in skin diseases. *The Journal of dermatology*. 1993;20(1):21-4.
37. Seo HM, Kim YH, Lee JH, Kim JS, Park YM, Lee JY. Serum Zinc Status and Its Association with Allergic Sensitization: The Fifth Korea National Health and Nutrition Examination Survey. *Scientific Reports*. 2017;7.
38. Yamada T, Saunders T, Kuroda S, Sera K, Nakamura T, Takatsuji T, et al. Cohort study for prevention of atopic dermatitis using hair mineral contents. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)*. 2013;27(2):126-31.
39. Ewing CI, Gibbs AC, Ashcroft C, David TJ. Failure of oral zinc supplementation in atopic eczema. *Eur J Clin Nutr*. 1991;45(10):507-10.

40. Kim JE, Yoo SR, Jeong MG, Ko JY, Ro YS. Hair zinc levels and the efficacy of oral zinc supplementation in patients with atopic dermatitis. *Acta Derm Venereol*. 2014;94(5):558-62.
41. Bailey RL, West KP, Jr., Black RE. The epidemiology of global micronutrient deficiencies. *Annals of nutrition & metabolism*. 2015;66 Suppl 2:22-33.
42. Illi S, von Mutius E, Lau S, Nickel R, Gruber C, Niggemann B, et al. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *The Journal of allergy and clinical immunology*. 2004;113(5):925-31.
43. Williams H, Stewart A, von Mutius E, Cookson W, Anderson HR. Is eczema really on the increase worldwide? *The Journal of allergy and clinical immunology*. 2008;121(4):947-54.e15.
44. Bel-Serrat S, Stammers AL, Warthon-Medina M, Moran VH, Iglesia-Altaba I, Hermoso M, et al. Factors that affect zinc bioavailability and losses in adult and elderly populations. *Nutr Rev*. 2014;72(5):334-52.
45. Vaghri Z, Barr S, Wong H, Chapman G, Hertzman C. Age-based differences in hair zinc of Vancouver preschoolers. *Biological trace element research*. 2008;126 Suppl 1:S21-30.
46. Xiang L, Sunesara I, Rehm KE, Marshall GD, Jr. Hair Cortisol Concentrations Are Associated with Hair Growth Rate. *Neuroimmunomodulation*. 2016;23(5-6):287-94.
47. Zalewski PD. Zinc metabolism in the airway: basic mechanisms and drug targets. *Current opinion in pharmacology*. 2006;6(3):237-43.
48. Carneiro MF, Rhoden CR, Amantea SL, Barbosa F, Jr. Low concentrations of selenium and zinc in nails are associated with childhood asthma. *Biological trace element research*. 2011;144(1-3):244-52.
49. de Cassia Ribeiro-Silva R, Fiaccone RL, Barreto ML, da Silva LA, Santos LF, Alcantara-Neves NM. The prevalence of wheezing and its association with serum zinc concentration in children and adolescents in Brazil. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)*. 2014;28(3):293-7.
50. Khanbabaee G, Omidian A, Imanzadeh F, Adibeshgh F, Ashayeripanah M, Rezaei N. Serum level of zinc in asthmatic patients: a case-control study. *Allergol Immunopathol (Madr)*. 2014;42(1):19-21.
51. Razi CH, Akelma AZ, Akin O, Kocak M, Ozdemir O, Celik A, et al. Hair zinc and selenium levels in children with recurrent wheezing. *Pediatr Pulmonol*. 2012;47(12):1185-91.
52. Uysalol M, Uysalol EP, Yilmaz Y, Parlakgul G, Ozden TA, Ertem HV, et al. Serum level of vitamin D and trace elements in children with recurrent wheezing: a cross-sectional study. *BMC pediatrics*. 2014;14:270.
53. Arik Yilmaz E, Ozmen S, Bostanci I, Misirlioglu ED, Ertan U. Erythrocyte zinc levels in children with bronchial asthma. *Pediatr Pulmonol*. 2011;46(12):1189-93.
54. Picado C, Deulofeu R, Lleonaart R, Agusti M, Mullol J, Quinto L, et al. Dietary micronutrients/antioxidants and their relationship with bronchial asthma severity. *Allergy*. 2001;56(1):43-9.
55. Urushidate S, Matsuzaka M, Okubo N, Iwasaki H, Hasebe T, Tsuya R, et al. Association between concentration of trace elements in serum and bronchial asthma among Japanese general population. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)*. 2010;24(4):236-42.
56. van Oeffelen AA, Bekkers MB, Smit HA, Kerkhof M, Koppelman GH, Haveman-Nies A, et al. Serum micronutrient concentrations and childhood asthma: the PIAMA birth cohort study. *Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology*. 2011;22(8):784-93.
57. Sagdic A, Sener O, Bulucu F, Karadurmus N, Ozel HE, Yamanel L, et al. Oxidative stress status and plasma trace elements in patients with asthma or allergic rhinitis. *Allergol Immunopathol (Madr)*. 2011;39(4):200-5.

58. Miyake Y, Sasaki S, Ohya Y, Miyamoto S, Matsunaga I, Yoshida T, et al. Dietary intake of seaweed and minerals and prevalence of allergic rhinitis in Japanese pregnant females: baseline data from the Osaka Maternal and Child Health Study. *Annals of epidemiology*. 2006;16(8):614-21.

Table 1. Main characteristics of observational studies included in the review**Case control studies**

Author (year)	Country of origin	Children¹ , adults or both	Mean age, years unless specified	n, cases	n, total	Males (%)	Type of control	Method of zinc determination	Method of AD determination	Matching or adjustment factors
Amin (2015)	Bangladesh	Both	Cases: 28,33 Controls: 28.86	65	130	Cases: 46.2 Controls: 61.5	Healthy	AAS ²	Dermatologist	Age
David (1984)	United Kingdom	Children	-	65	144	-	Hospital	AAS	-	None
David (1990)	United Kingdom	Children	Cases: 3,14 Controls: 9.78	134	246	-	Hospital	AAS	-	None
Di Toro (1987)	Italy	Children	Cases: 78 months Controls: 92 months	21	40	Cases: 66.7 Controls: 63.2	Healthy	PIXE ³	History, skin prick test, IgE levels	Age; income
el-Kholy (1990)	Egypt	Children	-	18	38	Cases: 55.6 Controls: 60.0	Healthy	AAS	History, examination and IgE levels	Age; sex; socio-economic status

Case control studies

Author (year)	Country of origin	Children ¹ , adults or both	Mean age, years unless specified	n, cases	n, total	Males (%)	Type of control	Method of zinc determination	Method of AD determination	Matching or adjustment factors
Hinks (1986)	United Kingdom	Adults	Cases: 39 Controls: 38.9	24	72	Cases: 50.0 Controls: 50.0	Healthy	AAS	-	Age Sex Smoking
Hon (2012)	Hong Kong	Children	Cases: 9,9 Controls: 11.6	119	162	Cases: 52,9 Controls: 65.1	Hospital	ICP-MS ⁴	Hanifin and Rajka's criteria	None
Hon (2010)	Hong Kong	Children	Cases: 9,9 Controls: 11.5	110	151	Cases: 51.8 Controls: 65.9	Hospital	ICP-MS	Hanifin and Rajka's criteria	None
Karabacak (2016)	Turkey	Both	Cases: 17,9 Controls: 16.7	67	116	Cases: 49.3 Controls: 49.0	Healthy	AAS	Hanifin and Rajka's criteria	None
Kim (2014)	South Korea	Children	Cases: 6,21 Controls: 6.74	58	101	Cases: 48.3 Controls: 46.5	Healthy	ICP-MS	Hanifin and Rajka's criteria	Age Sex
Tasaki (1993)	Japan	Adults	Cases: - Controls 31.3	11	59	-	Healthy	AAS	-	None
Toyran	Turkey	Children	Cases: 27	92	162	Cases: 63.0	Healthy	Randox kit spectro-	Consensus	

Case control studies

Author (year)	Country of origin	Children ¹ , adults or both	Mean age, years unless specified	n, cases	n, total	Males (%)	Type of control	Method of zinc determination	Method of AD determination	Matching or adjustment factors
(2012)			months Controls: 22.7 months			Controls 52.9		photometry	conference criteria	Age Sex

Cross sectional and cohort studies

Study (year)	Country of origin	Children, adults or both	Mean age, years unless specified	n, exposed	n, total	Males (%)	Definition of exposure	Method of zinc determination	Method of AD determination	Matching or adjustment factors
Seo (2017)	South Korea	Adults	Exposed (hypozincaemia) : 44,8 Unexposed: 44.9	554	1867	Exposed: 35,6 Unexposed: 54.9	Serum zinc <120µg/dL	ICP-MS	Japanese Dermatologic Society Diagnostic Criteria	None
Yamada (2013)	Japan	Infants	10 months	-	834	-	-	PIXE	Japanese Dermatologic Society Diagnostic Criteria	Family history of AD

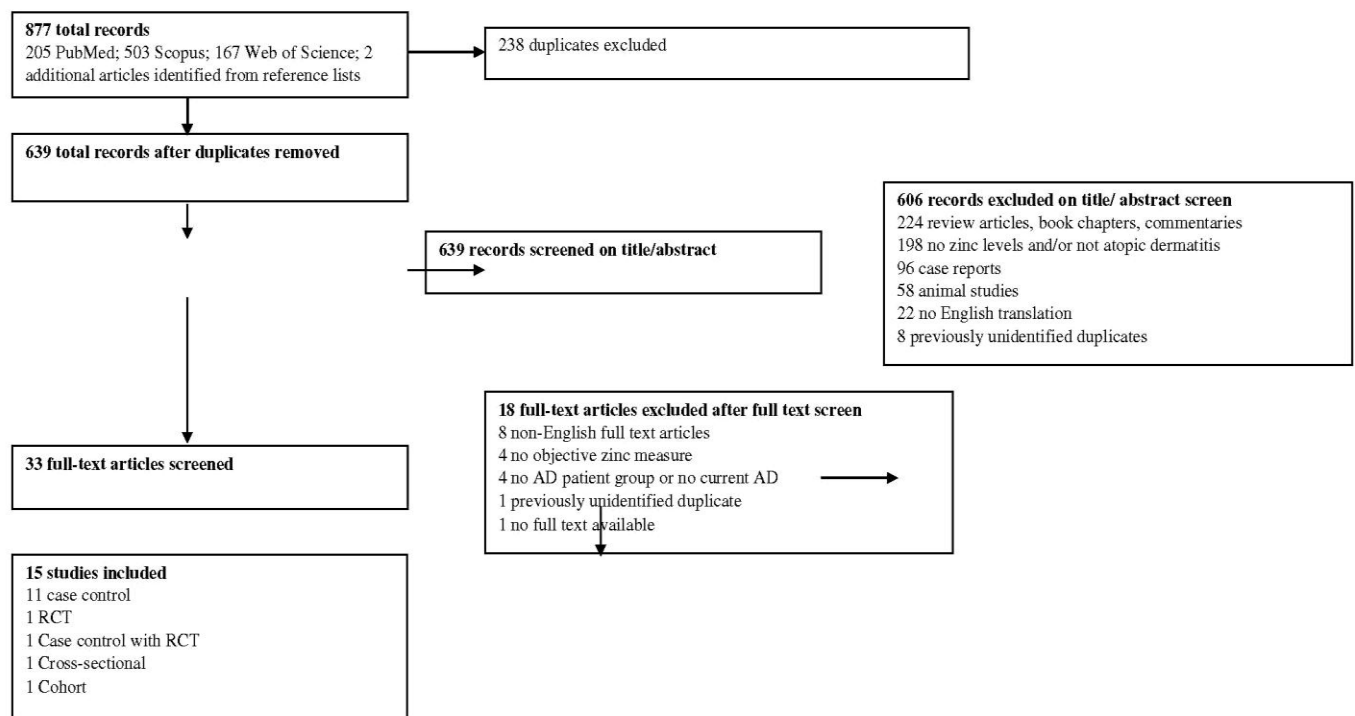
¹Children defined as <18 years of age

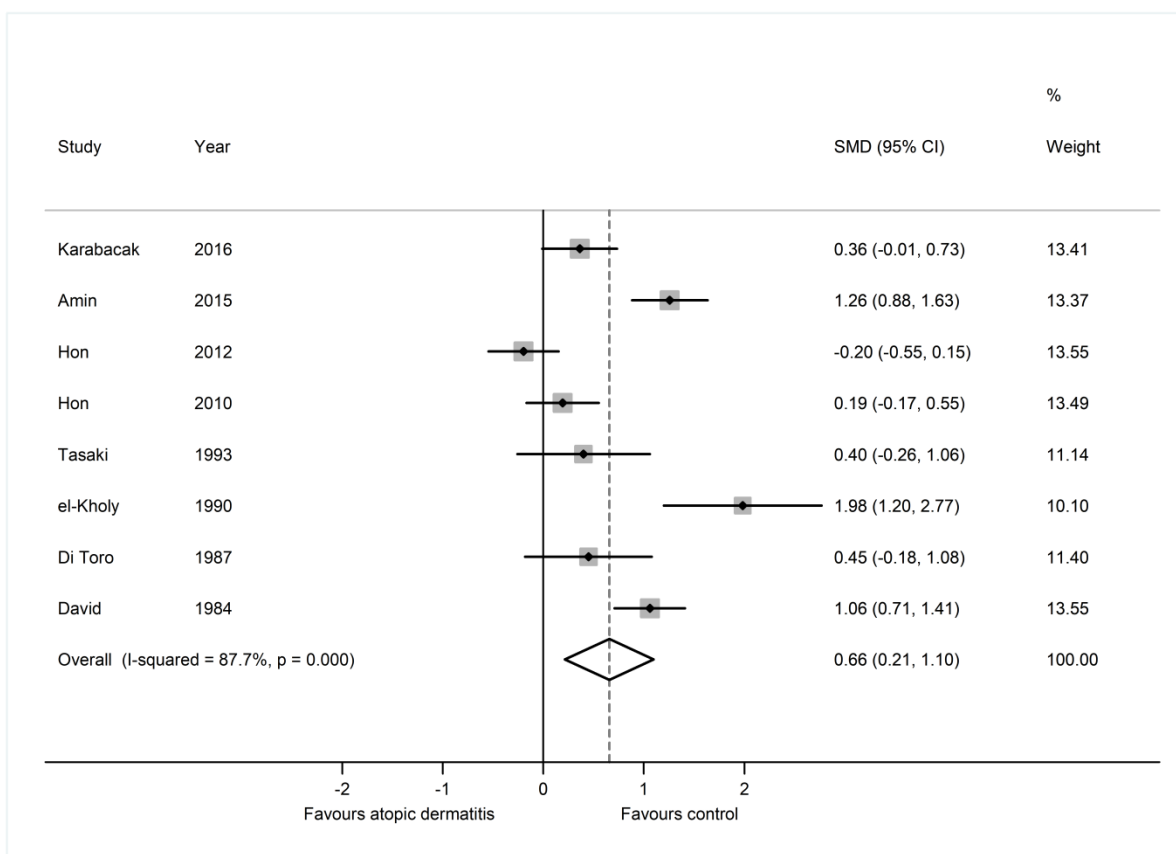
²AS: atomic absorption spectrometry

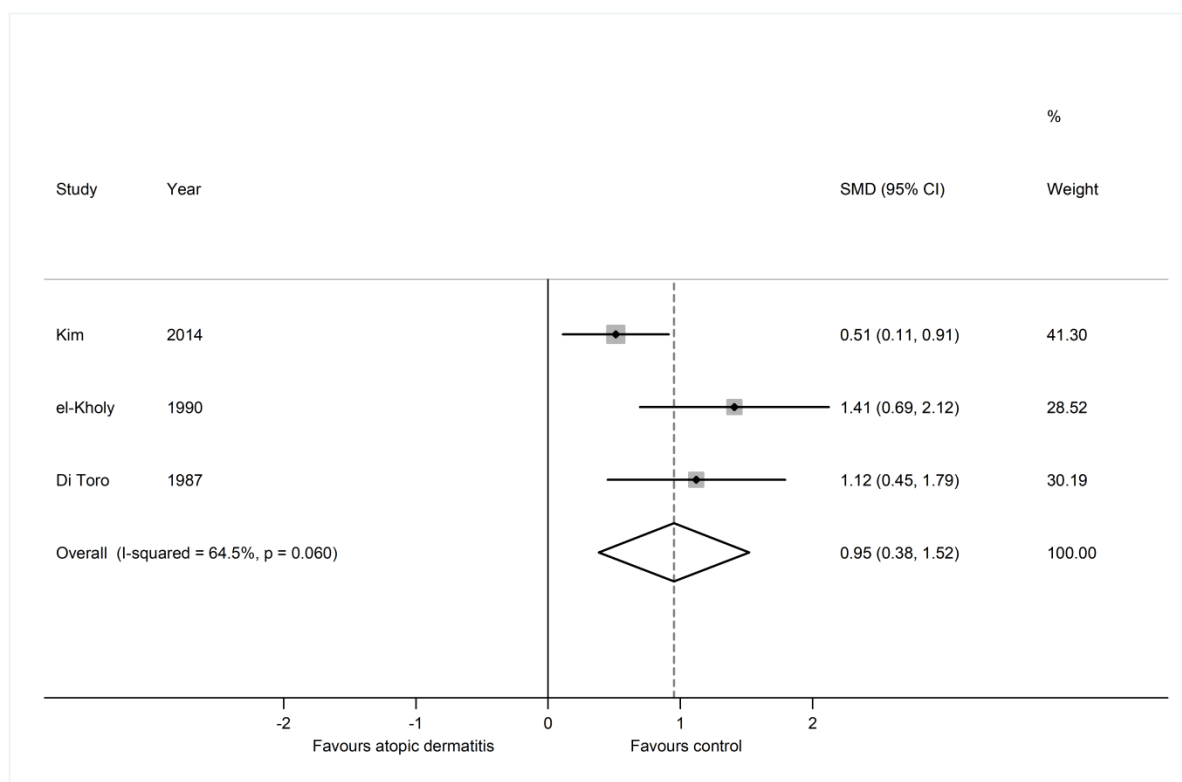
³PIXE: proton induced x-ray emission

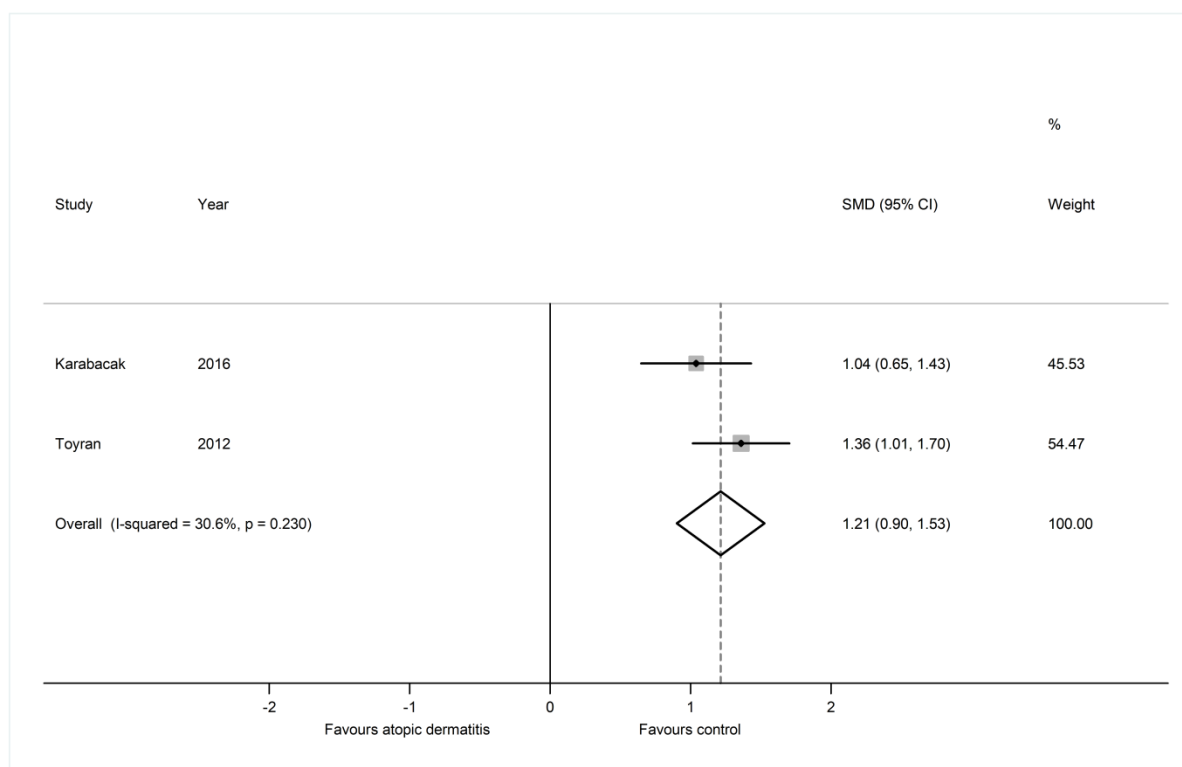
⁴ICP-MS: inductively coupled plasma mass spectrometry

Figure 1. Study selection process









Supplementary Table 1. Systematic search strategy

1) PubMed: ("Dermatitis, Atopic"[Mesh] OR "Eczema"[Mesh] OR eczema*[tiab] OR atopic dermat*[tiab]) AND ("Zinc"[Mesh] OR zinc[tiab])
2) Scopus: ((TITLE-ABS-KEY("atopic dermat*")) OR (TITLE-ABS-KEY("eczema")))) AND (TITLE-ABS-KEY("zinc"))
3) Web of Science: (TS="atopic dermat*" OR TS="eczema") AND (TS="zinc")

Supplementary Table 2. Quality of observational studies

Study	Selection	Comparability	Exposure/ Outcome ^a	Total
Case control studies ^b				
Amin (2015)	**	*	**	5
David (1984)	*	*	**	4
David (1990)	*		**	3
Di Toro (1987)	**	**	**	6
el-Kholy (1990)	**	**	**	6
Hinks (1986)	*	**	**	5
Hon (2012)	**		**	4
Hon (2010)	**		**	4
Karabacak (2016)	**	**	**	6
Kim (2014)	**	**	**	6
Tasaki (1993)	*		**	3
Toyran (2012)	**	**	**	6
Cross sectional studies ^c				
Seo (2017)	*****		**	6
Cohort studies ^c				
Yamada (2013)	*****	*	**	7

^a The third category examines assessment of exposure for case control studies and assessment of outcome of cross sectional and cohort studies.

^b Case control studies can obtain a maximum of 4, 2, and 3 stars for selection, comparability and exposure respectively. The maximum total score is thus 9.

^c Cross sectional and cohort studies can obtain a maximum of 5, 2, and 3 stars for selection, comparability and exposure respectively. The maximum total score is thus 10.

Supplementary Table 3. Quality of RCTs

Quality domain	Ewing (1991)	Kim (2014)
Sequence generation	high risk	high risk
Allocation concealment	high risk	high risk
Blinding of participants	low risk	high risk
Blinding of personnel and outcome assessors	low risk	low risk
Incomplete outcome data	low risk	unclear
Selective outcome reporting	low risk	low risk

Supplementary Table 4. Results for studies comparing mean zinc concentrations between participants with AD and controls.

Study (year)	Substrate for zinc measurement	Units	n cases	n controls	Zn cases; mean (SD)	Zn controls; mean (SD)
Karabacak (2016)	Erythrocyte	µg/g	67	49	34,4 (6,1)	40,4 (5,3)
Toyran (2012)	Erythrocyte	µg/dL	92	70	823,9 (120,9)	1040,9 (199,7)
Di Toro (1987)	Hair	µg/g	21	19	99 (45,83)*	147 (39,23)*
el-Kholy (1990)	Hair	µg/g	18	20	164,8 (23.6)	194,5 (18.6)
Kim (2014)	Hair	µg/g	58	43	113,10 (33,36)	130,90 (36,63)
Hinks (1986)	Plasma	µmol/L	24	48	13,5 (1,8)	12,9 (1,9)
Amin (2015)	Serum	mg/L	65	65	0,30 (0,16)*	0,75 (0,48)*
David (1984)	Serum	µmol/L	65	79	11,4 (2,0)	13,7 (2,3)
Di Toro (1987)	Serum	µmol/L	21	19	14,8 (2,75)*	16,2 (3,49)*
el-Kholy (1990)	Serum	µg/100ml	18	20	65,9 (11,7)	88,4 (11,0)
Hon (2012)	Serum	µmol/L	119	43	9,0 (2,5)	8,5 (2,7)
Hon (2010)	Serum	µM	110	41	8,58 (2,78)	9,10 (2,55)
Karabacak (2016)	Serum	µg/g	67	49	89,9 (16,8)	96,1 (17,6)
Tasaki (1993)	Serum	-.	11	48	85,0 (8,5)	90,8 (15,5)

*Standard deviation calculated from standard error and sample size provided in original article

Supplementary Table 5. Meta-regression for mean serum zinc levels comparing participants with AD to controls.

Trial characteristic	Number of studies	SMD (95% CI)	I ² (%)	P _{heterogeneity}
Study quality				
≥6 stars	3	0.88 (-0.00-1.76)	85.5	0.56
<6 stars	5	0.55 (-0.04-1.13)	90.7	
Region				
Other	5	0.69 (-0.01-1.39)	91.6	0.93
Europe	3	0.65 (0.15-1.14)	74.5	
Matched				
Yes	5	0.55 (-0.04-1.13)	90.7	0.56
No	3	0.88 (-0.00-1.76)	85.5	
Age				
Children	5	0.65 (0.01-1.29)	90.3	0.95
Other	3	0.69 (0.05-1.34)	83.7	
Sample size				
≥100	5	0.53 (-0.00-1.07)	90.9	0.50
<100	3	0.92 (-0.03-1.87)	82.4	
Type of controls				
Healthy	5	0.86 (0.31 – 1.42)	82.1%	0.33
Other	3	0.35 (-0.38-1.09)	92.3%	