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REVIEW

Association between serum zinc levels and recurrent aphthous stomatitis: a meta-analysis with trial sequential analysis

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Abstract

Objectives The present meta-analysis sought to investigate the potential association between zinc levels and recurrent aphthous stomatitis (RAS).

Methods A comprehensive search of online databases (PubMed, Scopus, Web of Science, and China National Knowledge Infrastructure (CNKI)) was conducted to identify all English and Chinese studies published up to August 2020. All case-control studies that assessed plasma/serum zinc levels were eligible for inclusion. Data were analyzed using Comprehensive Meta-Analysis software version 2.2.046 (Biostat, Englewood, NJ, USA). Trial sequential analysis (TSA) was conducted with the trial sequential analysis program.

Results Nineteen case-control studies, involving 1079 RAS cases and 965 controls, were included in the meta-analysis. The pooled results of 19 studies showed that zinc level was significantly lower in RAS patients than in healthy controls (weighted difference in means = − 21.092, 95% CI − 26.695 to − 15.490, $I^2 = 95.375\%$, $P < 0.001$). Upon subgroup analysis by geographic distribution of the sample (Chinese vs. others), the association remained significant in each individual subgroup, although the association was more pronounced among Chinese populations. TSA indicated that the current studies surpassed the required information size, confirming that the differences were reliable.

Conclusion The results suggest a significant association between low serum zinc levels and the occurrence of RAS. Although TSA confirmed a solid conclusion, conducting large-scale studies with the highest standards of quality is encouraged.

Clinical relevance Determining zinc levels should be considered in diagnosis, management, and prevention of RAS.

Keywords Aphthous stomatitis · Zinc · Association · Meta-analysis

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Introduction

Recurrent aphthous stomatitis (RAS) or recurrent aphthous ulcers is a very common painful disease of oral mucosa, affecting up to 25% of the general population [1–4]. It is characterized by recurrent single or multiple ulcers affecting mainly the non-keratinized mucosa [5]. On the basis of the clinical presentation, RAS is classified as minor, major, and herpetiform [1–3]. The minor form is the commonest type accounting for around 85% of all RAS cases; it presents as a small well-circumscribed ulcer of less than 1 cm in diameter that heals spontaneously within 7–10 days [2, 4]. Although self-limiting, RAS is quite annoying that can interfere with the daily functions such as eating and speaking, thus impairing the patient's quality of life [5, 6]. The treatment of RAS is quite challenging, and as yet, there is no definitive cure; the primary goal of RAS management is to reduce pain, promote healing, and reduce the frequency of episodes [4–7].

Despite the extensive research on RAS, the exact etiopathogenesis is still unclear [8]. However, some local and systemic predisposing factors have been suggested. These include genetic predisposition, local trauma, stress, nutritional deficiencies, immunological disturbances, systemic diseases, and vitamin deficiency [5, 8–11].

Nutritional deficiencies of iron and certain vitamins such as B12, B2, D, and B9 (folic acid) have been implicated in pathogenesis of RAS [9, 10]. Recent data suggest that even the trace elements such as zinc may have a role in pathogenesis of RAS [12–14]. In fact, zinc is a vital micronutrient that plays pivotal roles in cell growth/reproduction, collagen synthesis, and wound healing [15]. Additionally, zinc has been reported to have an essential role in regulating the immune system, including innate and adaptive immune responses [15]. Many studies have linked zinc deficiency with several disorders such as rheumatoid arthritis, vitiligo, ectopic dermatitis, and multiple sclerosis [15–18]. In this regard, several observational studies have investigated the potential association between zinc levels and RAS, and conflicting results were reported [12, 13, 19–22]. Several studies found a significant association between zinc deficiency and the development of RAS [12–14, 20, 23], whereas a few others did not find any association [19, 21, 22]. It seems that there is some controversy regarding the potential role of zinc in pathogenesis of RAS. Therefore, the present meta-analysis sought to assess the available evidence on the potential association between zinc level and the occurrence of RAS.

Materials and methods

Study question

The current meta-analysis followed strictly the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [24]. The study was registered in the PROSPERO database (ID=CRD42020198559). The focused research question was "Is there any association between zinc deficiency and the occurrence of RAS?"

Eligibility criteria

This current systematic review included all observational studies that assessed the potential role of zinc deficiency in developing of RAS, and fulfilled the following criteria: (1) comprised RAS patients and control subjects, (2) RAS patients are systemically healthy ≥ 15 years old, (3) the main outcome measure reported quantitative zinc level (mean ± SD), and (4) the language was either English or Chinese. The following studies were excluded: (1) those without control group, (2) those without adequate quantitative data (mean ± SD), (3) studies that assessed other than serum/plasma zinc levels (e.g., hair), (4) case series, (5) case reports, (6) in vitro studies, and (7) review articles.

Search strategy

PubMed, Scopus, Web of Science, Google Scholar, and China National Knowledge Infrastructure (CNKI) databases/search engines were searched up to August 2020. The following key words were used: (Zinc OR "Zinc deficiency" OR "Zinc level" OR "Zinc levels" OR "serum Zinc" OR "plasma Zinc") AND ("recurrent aphthous stomatitis" OR "aphthous stomatitis" OR "recurrent aphthous ulcers" OR "recurrent aphthous ulcer" OR "aphthous ulcers" OR "aphthous ulcer" OR "canker sore"). The Chinese translations of these key words were used for the search in the CNKI database. .

Data extraction

The eligible studies were processed for data extraction. Two authors (AS and AA) independently extracted the necessary information from the English studies, and other two authors with good experience the Chinese language (HA and AA) independently extracted the necessary information from the Chinese studies. The following data were extracted from each study: authors and year of the article, country, study design, number of RAS patients and control subjects, gender, age, clinical type of RAS, diagnostic criteria of RAS, assessment methods, and serum/plasma zinc levels.

Quality assessment

The quality (risk of bias) of the included studies was assessed using Newcastle-Ottawa Scale (NOS) [25], which is based on three major components: selection of the study groups (0–4 stars), comparability of cases and controls by controlling for relevant factors (0–2 stars), and exposure (0–3 stars). The

overall quality of a given study is rated as either high quality, 7 stars or more; moderate quality, 4–6 stars; or poor quality, 0–3 stars. Two independent investigators (AS and AA) assessed the risk of bias of the English studies, and another group of tow investigators (HA and AA) assessed the risk of bias of the Chinese studies; disagreement between the two investigators, if any, was solved via discussion and/or by consulting a third reviewer (EH) until consensus is reached.

Statistical analyses

The most widely used unit of measurement of serum zinc levels is μg/dL. Means and standard deviations of serum zinc levels which presented otherwise were converted to μg/dL in the following website: http://unitslab.com/node/142. Serum zinc levels, which were presented in unusual unit of measurements or, upon conversion, the values were extraordinary, were excluded. Data were analyzed using Comprehensive Meta-Analysis software version 2.2.046 (Biostat, Englewood, NJ, USA). The weighted difference in means between the two groups along with the 95% confidence intervals (CI) was calculated. Heterogeneity was identified using chi-squared test and the I^2 statistics. "The random effects model" was used in case the heterogeneity was significant ($I^2 > 50\%$); otherwise, "the fixed effects model" was applied. A P value less than 0.05 was considered statistically significant.

Trial sequential analysis

The main aim of rrial sequential analysis (TSA) is to control for increased random error that may arise in meta-analyses. Simply, TSA generates monitoring boundaries and estimates an optimal sample size in cumulative meta-analyses, creating thresholds for declaring significance [26]. Every time a new study is included in a meta-analysis, the significance is tested, along with estimating the confidence intervals. In line with that, the cumulative Z curves that represent the findings of the data are created. When the Z curves surpass the monitoring boundary, the level of evidence is said to be sufficient and further trials are deemed futile. Hence, once one of the boundaries or the optimal sample size in cumulative meta-analyses is reached, the conclusion is said to be conclusive and confirming, and no further trials are needed. Otherwise, more trials are needed to clarify the conclusion [27]. In our study, we used two-sided trial sequential monitoring boundary type, and calculated the required information size (RIS) based on $\alpha = 0.05$ and $\beta = 0.20$. The mean differences and effect sizes were calculated from the included studies. The software TSA version 0.9.5.10 beta was used for the analysis (Copenhagen Trial Unit, Centre for Clinical Intervention Research, www.ctu.dk/tsa).

Publication bias

Publication bias was assessed using funnel plot and Egger's test. The funnel plot was conducted using Review Manager 5.3 software (RevMan 5.3, The Cochrane Collaboration, Copenhagen, Denmark), while the Egger's test was conducted using Stata software programs for Windows (Stata Corporation, version 15.1, College Station, TX, USA).

Results

Study selection

The search strategy is illustrated in Fig. 1. A total of 708 studies were identified; 120 studies were duplicates and were thus removed. The titles and abstracts of the remaining 588 studies were screened. Of these, 506 were found to be irrelevant and were thus excluded. The full texts of the remaining 82 articles were screened, and 63 studies were excluded due to various reasons: lack of control group, missing data, studies conducted on children, studies published in languages other than English or Chinese, use of unclear unit of measurement, case reports, and reviews. Eventually, 19 case-control studies [12–14, 19–23, 28–38] were included in the current meta-analysis.

General characteristics of the included studies

Table 1 presents the characteristics of the included studies. A total of 19 case-control studies (in English language, $n = 7$; in Chinese, $n = 12$) were included in the present meta-analysis [12–14, 19–23, 28–38]. The sample comprised 2044 subjects (1079 RAS cases and 965 controls). Thirteen studies [12, 23, 28–38] were conducted in China, 3 in Turkey [13, 14, 22], one in Iran [20], one in India [19], and one in Poland [21]. The main age of the included subjects ranged from 15 to 68 years, with around 40% ($n = 812$) of the subjects being females. All studies assessed serum zinc levels. The assay method of zinc level varied among studies, although the majority used atomic absorption spectrometer method. All studies included healthy RAS patients and control individuals with no history of systemic diseases.

Meta-analysis results

All studies ($n = 19$) were included in the meta-analysis. Fifteen of the included studies [12–14, 20, 23, 28, 30–38] found a significant association between low zinc levels and RAS, whereas the remaining 4 studies [19, 21, 22, 29] did not find such association (Table 1).

The results of the pooled 19 studies revealed that zinc level was significantly lower in RAS patients than in healthy controls (weighted difference in means = − 21.092, 95% CI − 26.695 to − 15.490, $I^2 = 95.375\%$, $P < 0.001$; Fig. 2).

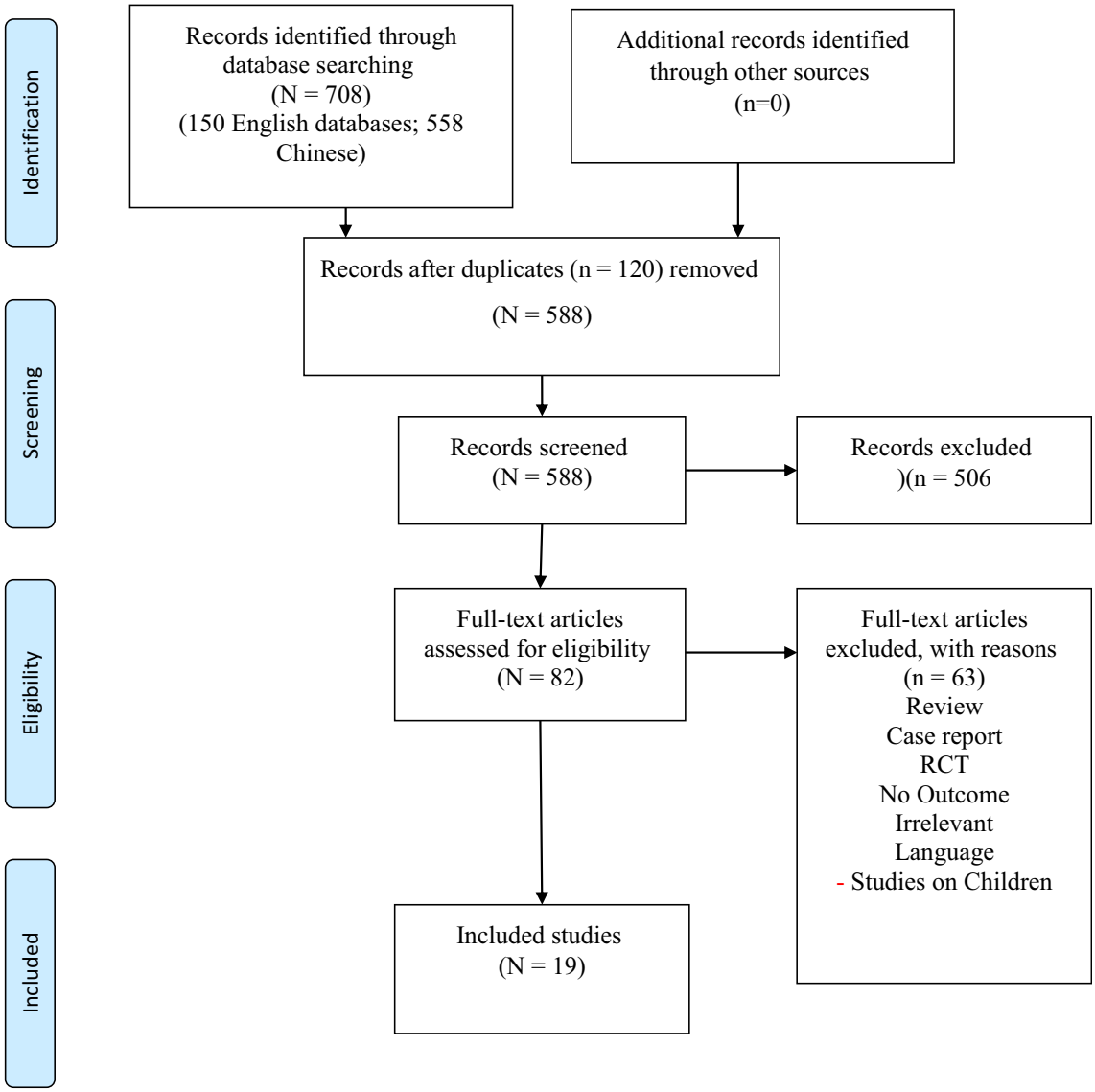

Fig. 1 Flow chart of methodology according to PRISMA guidelines

Upon subgroup analyses by geographical distribution of the samples (Chinese vs. others), the results remained significant, although the relationship was more pronounced among Chinese populations (weighted difference in means = − 24.279, 95% CI − 30.828 to − 17.731, I^2 = 95.733%, P < 0.001) compared with other nationalities (weighted difference in means = − 13.768, 95% CI − 25.824 to − 1.705, I^2 = 94.395%, P = 0.025) (Supplementary figure 1).

Trial sequential analysis

TSA showed that the cumulative Z curves crossed the conventional boundary and the trial sequential monitoring boundary, and surpassed the required information size (n = 310) as well (Fig. 3). Thus, the conclusion is reliable and confirming, and further trials are not needed.

Publication bias

On the basis of visualization of Funnel plot (Supplementary Figure 2), there was no evidence of asymmetrical pattern with the included data, indicating no publication bias. This was further confirmed with the quantitative analysis using Egger's test (P = 0.982).

Quality of the included studies

The results of the quality assessment of the included studies are presented in Table 2. Two [21, 33] of the included studies were of high quality, 14 studies [12–14, 20, 22, 23, 28, 30, 31, 34–38] were of moderate quality, and the other three studies [19, 29, 32] were of low quality (Table 2).

Table 1 General characteristics of the included studies

Author and country	Sample size Cases/ controls	Gender M/F (age)	Type of RAS	Diagnostic criteria of RAS	Blood sample	Method of zinc assessment	Main outcomes
Arora et al. (2002) India	15/24	NA	NA	History clinically	Serum	Atomic absorption	No significant difference between the two groups ($P > 0.05$)
Özler (2014) Turkey	25/25	M/F 25/25 Age 20–40	Minor	History clinically	Serum	Flame atomic absorption	Significantly lower zinc levels in RAS patients than controls
Khademi and Shaikhiany (2006)	44/44	M/F 28/60 Age NA	Minor Major Herptiform	Clinical examination	Serum	Atomic Absorption	Significantly lower zinc levels in RAS patients than controls
Ozturk et al. (2013) Turkey	33/30	M/F 20/43 Age 15–69	Minor	History clinically	Serum	Atomic absorption	Significantly lower zinc levels in RAS patients than controls
Tamer and Avcı (2019) Turkey	20/20	M/F 11/29 Age 18–60	NA	NA	Serum	NA	No significant difference between the two groups ($P > 0.05$)
Bao et al. (2016) China	156/115	NA	Minor	History clinically	Serum	Colorimetric method zinc assay kit	Significantly lower zinc levels in RAS patients than controls
Ślebioda et al. (2017) Poland	75/72	M/F 48/99 Age 35.08	Minor Major Herptiform	History clinically	Serum	Flame atomic absorption	No difference between the two groups ($P > 0.05$)
Li et al. (2005) China	50/40	M/F 40/50 Age 18–60	NA	NA	Plasma	Three-electrode plasma atomic emission spectrometer	Significantly lower zinc levels in RAS patients than controls
Ming et al. (1997) China	50/50	M/F 40/60 Age 16–33	NA	NA	Serum	Pyrazolyl colorimetric method.	Significantly lower zinc levels in RAS patients than controls
Li et al. (2010) China	28/28	M/F 24/32 Age 19–58	NA	NA	Serum	NA	Significantly lower zinc levels in RAS patients than controls
Cai et al. (1995) China	76/50	RAS: M/F 36/40 Age 27–59	NA	NA	Serum	Atomic absorption spectrophotometer	No significant difference between the two groups ($P > 0.05$)
Liu et al. (1995) China	42/41	M/F 49/34 Age 15–68	NA	NA	Serum	jp-2 polarography for atomic absorption	Significantly lower zinc levels in RAS patients than controls
Song et al. (2002) China	26/20	M/F 22/24 Age 20–55	NA	NA	Serum	Atomic absorption spectrometer	Significantly lower zinc levels in RAS patients than controls
Li et al. (2016) China	65/65	M/F 57/73 Age 18–57	NA	NA	Serum	Centrifugation for serum zinc detection	Significantly lower zinc levels in RAS patients than controls
Sun et al. (1994) China	51/50	M/F 52/49 Age 24–68	NA	NA	Serum	Three-electrode plasma atomic emission	Significantly lower zinc levels in RAS patients than controls
Liu et al. (2010) China	83/83	M/F 88/78 Age 23–32	NA	NA	Serum	Atomic absorption spectrometer	Significantly lower zinc levels in RAS patients than controls
Tong et al. (2018) China	76/76	M/F 77/75 Age 21–60	NA	NA	Serum	Beckman AU5831 automatic biochemical analyzer	Significantly lower zinc levels in RAS patients than controls
Song et al. (2016) China	67/30	M/F 56/41 Age 21–59	54 Mild and 13 severe cases	NA	Serum	CA-1500 automatic blood coagulometer	Significantly lower zinc levels in RAS patients than controls
Zhang et al. (2018) China	97/102	M/F 92/107 Age RAS 31.38 Controls 29.83	Minor	NA	Serum	spectrophotometer (Alpha-1106)	Significantly lower zinc levels in RAS patients than controls

RAS recurrent apthous stomatitis, *M* male, *F* female, *NA* not available

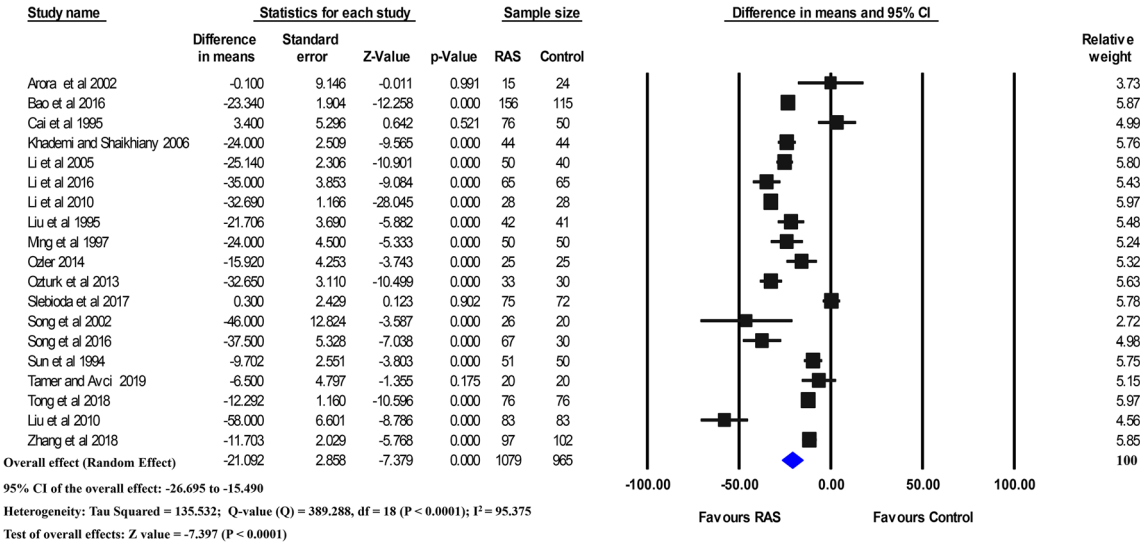

Fig. 2 Forest plot of the Meta-analysis

Discussion

RAS is a very common oral lesion of unclear etiology, what makes its therapy a challenge [4, 5, 39]. Many studies have investigated the potential association between zinc levels and RAS, and reported conflicting results [12, 13, 20]. Hence, the present meta-analysis was performed to answer the focused question: Is there any association between zinc levels and the development of RAS? The results of the pooled 19 studies showed that patients with RAS have significantly lower zinc levels than healthy controls. Additionally, upon subgroup analysis by geographical distribution of the sample, the results

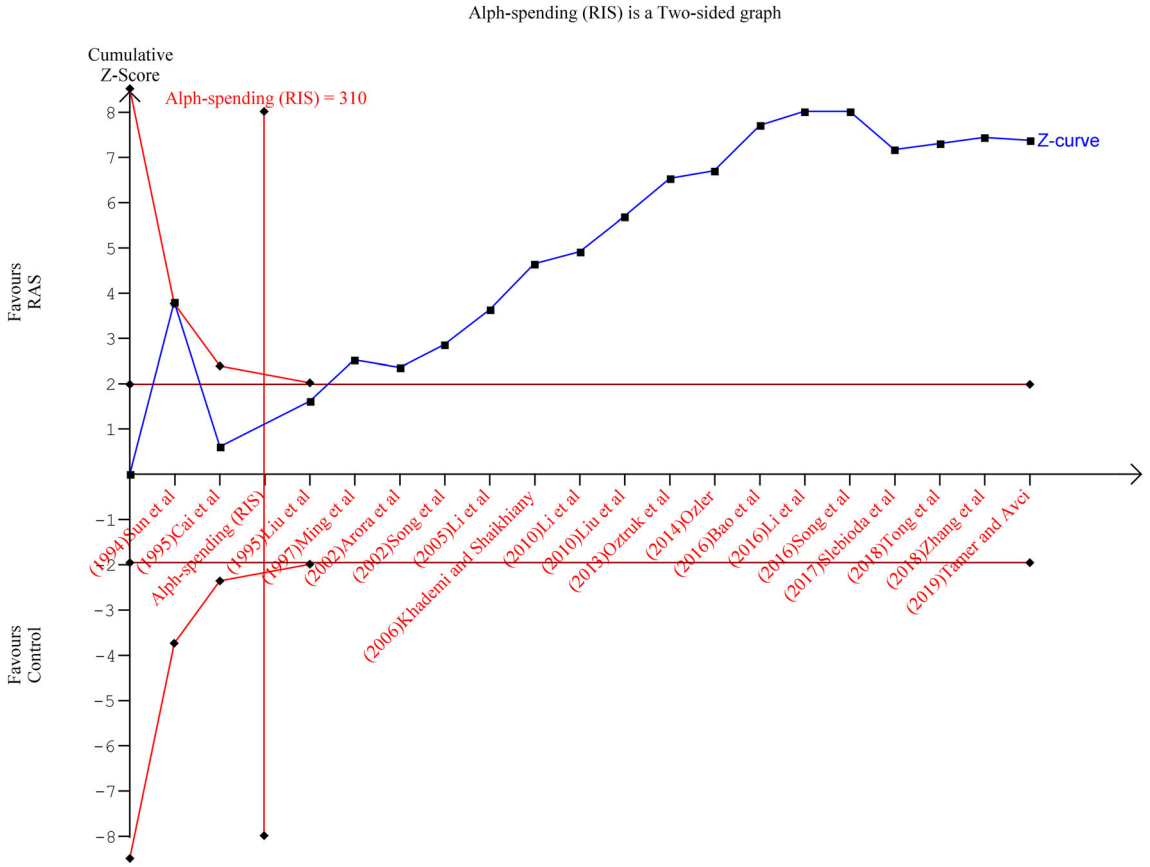

Fig. 3 Trial sequential analysis

Table 2 Results of the quality assessment using NOS for case-control studies

Study	Selection	Comparability	Exposure	Total score
Arora et al. (2002)	***		*	4
Özler et al. (2014)	***	**	*	6
Khademi and Shaikhiany	****		*	5
Ozturk et al. (2013)	***	**	*	6
Tamer and Avcı (2019)	***	*	*	5
Bao et al. (2016)	***	**	*	6
Ślebioda et al. (2017)	****	**	**	8
Li et al. (2005)	****	*	**	7
Ming et al. (1997)	***	*	**	6
Li et al. (2010)	***	**	**	7
Cai et al. (1995)	**	-	**	4
Liu et al. (1995)	**	-	**	4
Song et al. (2002)	**	*	**	5
Li et al. (2016)	***	**	**	7
Sun et al. (1994)	***	*	**	6
Liu et al. (2010)	***	**	***	8
Tong et al. (2018)	***	*	**	6
Song et al. (2016)	***	*	**	6
Zhang et al. (2018)	***	-	**	5

remained significant, although the relationship was more pronounced among Chinese compared with other countries. The results of the current meta-analysis were further confirmed by TSA, which showed that the current sample size is surpassing the required one to draw a definitive conclusion (Fig. 3). This was true even when TSA was conducted for subgroups (based on geographical differences) separately, although this was more pronounced for Chinese studies. Nevertheless, conducting well-designed, large-scale studies targeting different populations is still encouraged [26].

The results of the present meta-analysis corroborate previous systematic reviews that reported a significant association between zinc deficiency and several autoimmune diseases such as ectopic dermatitis, rheumatoid arthritis, multiple sclerosis, and vitiligo [16–18]. Zinc is the second most abundant trace element in the human body [40]; it has a vital role in regulating the innate and adaptive immune responses, cell cycle progression, and differentiation [15]. Although the precise etiopathogenesis of RAS is still uncertain, compelling evidence indicates that immunological dysfunction/deregulation is implicated [8]. Hence, considering the pivotal role of zinc in the immune system and the postulated immunological background of RAS, the potential role of zinc in the pathogenesis of RAS can be conceptualized.

In meta-analysis, it is known that the quality of the included studies is key for determining the level of evidence. For this purpose, the quality of all included studies was thoroughly scrutinized using NOS, a well-known quality appraisal tool for observational studies [25]. The NOS-based results revealed that apart from two studies, which showed high quality, most of the included studies showed some methodological flaws. Such methodological flaws may limit the results of the current study.

The present meta-analysis advocates a significant association between zinc deficiency and the risk of RAS. However, there are some limitations that should be considered when interpreting results of the present meta-analysis. The key limitation is that the evidence obtained is solely based on observational studies, and so the potential confounders might have not been controlled. Another important limitation is the marked heterogeneity among the included studies with respect to settings, methods of zinc measurements, type of RAS, gender, and age of participants. Nevertheless, despite these limitations, the study has several strengths that should be acknowledged. First, this is the first study that summarized the evidence on the association between zinc and RAS. Second, this meta-analysis included a relatively huge sample size, and included all potential studies in the English and Chinese literature. Finally, the results of the meta-analysis were further confirmed by secondary analysis using trial sequential analysis.

In conclusion, the present meta-analysis reveals a significant association of low serum zinc and the risk of RAS. Although TSA confirmed this conclusion, conducting well-designed, large-scale studies targeting different populations is highly recommended.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00784-020-03704-8.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This is a review article, and so no ethical approval is required.

Informed consent Not required for this type of study.

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