Systematic Review of Zinc Biomarkers and Esophageal Cancer Risk

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ABSTRACT

BACKGROUND

It is hypothesized that poor zinc nutritional status is associated with an increased risk of esophageal cancer (EC), but current evidence is contradictory. Since some factors may influence zinc absorption, its status may be better evaluated thorough biomarkers. The objectives of this study were to perform a systematic review on the association of zinc biomarkers with EC in observational studies and to evaluate the efficacy of zinc supplements in preventing EC in randomized trials.

METHODS

The MEDLINE database was searched in December 2013 for studies written in English with relevant keywords. Articles which met inclusion criteria were included in this study.

RESULTS

Eleven observational studies that measured zinc biomarkers and eight randomized trials which evaluated supplements containing zinc, met our inclusion criteria. The majority of studies suggested that higher zinc status was inversely associated with EC risk.

CONCLUSION

Most of the evidence for this hypothesis comes from case-control studies, which may introduce bias. Cohort studies are needed to establish whether poor zinc status is associated with increased risk for EC. Findings from trials are inconclusive as there is no data from single agent trials. However, the evidence is not still strong enough to conclude a protective role of zinc in EC.

KEYWORDS
Zinc; Esophageal cancer; Minerals; Systematic review

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INTRODUCTION

Zinc (Zn) is essential for the activity of more than 300 enzymes, immune function, and conformation of many transcription factors that control cell proliferation, apoptosis, and signaling.[1] Zn is available from all food groups, but some important dietary sources of Zn include red meat, poultry, fish, other seafood, legumes, nuts, whole grains, and dairy products.[2] However, the concentration of Zn in most foods is not inherent and the Zn content of foods depends on soil and water Zn concentrations or in the concentration in fodder. In addition, there are some physiologic factors such as age, genotype, and the quantity of Zn ingested, and the time over which Zn is ingested that may affect Zn absorption. Furthermore, the bioavailability of ingested Zn is dependent on the presence of phytate in foods, which inhibits Zn absorption.[3,4] For these reasons, dietary intake methods are likely inaccurate for estimating Zn deficiency or Zn exposure and observational studies of Zn status may benefit from the use of biomarkers such as hair, nail, serum or plasma Zn concentrations.

Zn deficiency adversely affects the immune system, increases oxidative stress, and increases the generation of inflammatory cytokines.[5] In animal models, a Zn deficient diet results in a precancerous condition in the upper digestive tract, including the esophagus[1] and enhances the effects of esophageal carcinogens (e.g., N-nitrosomethyl benzylamine)[6] by different mechanism including increased cell proliferation,[7] cyclin D1 over expression[8] and p53 deficiency.[9] Other mechanisms may include cyclooxygenase-2 (COX-2) over expression,[10] activating S100A[8] inflammation,[1] P450-dependent metabolism of nitrosamines,[11] and reduced alkyl guanine DNA methyltransferase activity.[12] Moreover, in rodents, Zn supplementation may affect tumor progression[13] by inducing apoptosis,[14,15] and reversing over expression of the S100A8.[16] In a rat model, a chronic Zn deficient diet induces a pro-tumorigenic micro RNA signature (miR-31 and miR-21) that fosters squamous cell carcinoma development.[17] However, the effect of Zn on esophageal cancer (EC) risk in humans is uncertain.[18-20]

EC is the eighth most common cancer with respect to incidence and the sixth most common cancer with respect to mortality worldwide.[21] EC is classified into two main types histologically: esophageal adenocarcinoma (EA) and esophageal squamous cell carcinoma (ESCC), each having different risk factors.[19] Numerous observational studies have investigated the association between Zn biomarkers measured in nails, hair, plasma, or serum and EC risk. Furthermore, several randomized trials have tested whether Zn supplementation (in combinations with other nutrients) reduced the incidence of EC. However, the totality of evidence has not been systematically reviewed.

The objective of the present study was to review the results from observational studies about the association of Zn status (using all biomarkers of Zn) with EC and results of clinical trials about the efficacy of Zn supplements in preventing EC.

MATERIALS AND METHODS

Data sources, search strategy, and selection criteria

MEDLINE database was searched for observational studies and randomized trials investigating the relationship between Zn and EC. The following Medical Subject Headings (MeSH) terms were applied [“esophag*” AND (“cancer” OR “tumor” OR “carcinoma” OR “adenocarcinoma” OR “neoplasm”)]; and were combined with each of the terms “zinc”, “zn”, “zinc gluconate”, “zinc sulfate”, “zinc acetate”, “zinc oxide”, “methalothionein”, and “zinc isotope”. The potentially relevant articles were included if the full paper had been obtained. No time restrictions were added. Studies were restricted to human studies and publications in English. References of identified articles and reviews were also searched for additional relevant articles.

We aimed to identify all observational and randomized trials that assessed the association of Zn with EC, either alone or combined with other nutrients, for preventing EC. The endpoint was EC, which was defined as any combination of EA and ESCC. Studies reporting only EC without the type of pathology were also included. Articles with the following criteria were excluded:

1- Not original research (reviews, editorials, non-research letters);
2- Case reports or case series;
3- Ecologic studies;
4- Studies lacking a biomarker of Zn status.

In the case of several reports on one outcome from the same population, the last publication was enrolled[22-26] (Figure1).

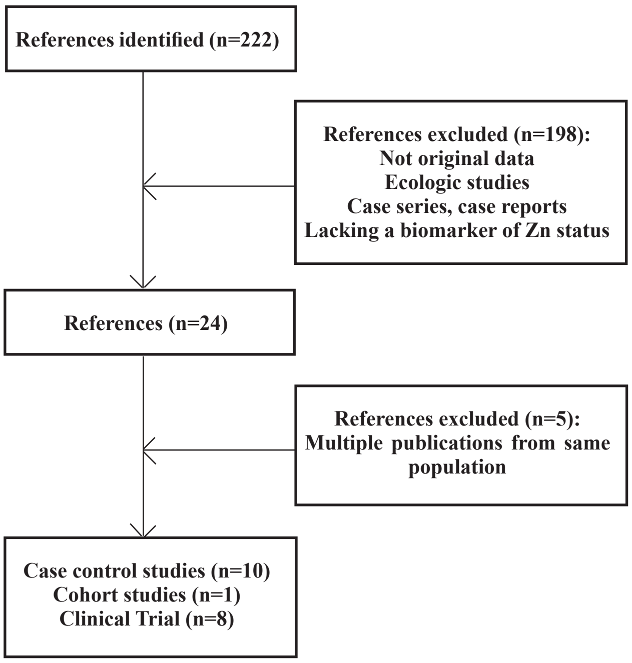

Fig.1: **Flow diagram of study selection process**

Data extraction and quality assessment

One investigator (MH) reviewed search results and extracted the study design, first author, year of publication, country, patient characteristics (sex and mean age), sample size, and the reported RR (OR) with 95% confidence intervals (CIs) for the highest versus lowest categories of Zn status from studies (table 1). The quality of observational studies was assessed according to the criteria used by Flores-Mateo et al.[27] (appendix 1), and the quality of randomized trials was assessed according to the criteria of Jadad et al.[28] (table 2).

RESULTS

Observational studies

Ten case-control studies[29-38] and one prospective cohort study[39] were included in the study (figure 1). The studies were published between 1983 and 2013 (table 1). Most studies were performed on participants from Asia. The number of participants varied between 27[36] and 358.[29] The quality scores varied widely (appendix 1). Most articles which had evaluated the association between EC and Zn examined ESCC, with a single study of EA and one EC, where histology was not specified. The single case-control study of EA found no association,[29] between Zn and the risk of EC while most studies on ESCC found an inverse association between Zn and the risk of EC (table 1).

Randomized trials

Eight trials[40-47] were included in this study, which were published between 1987 and 2013 (table 2). All trials used Zn combined with other vitamins or minerals. Zn doses were 22.5mg/d zinc oxide or 45mg/d zinc sulfate 33 or 50 mg zinc weekly. In two trials, the form of Zn was not specified. All trials were placebo-controlled and double-blinded. The length of intervention ranged from 13.5 months to 6 years, while some studies have included post-intervention follow-up of up to 20 years. All trials were performed in China and most of the reports came from the two Nutrition Intervention Trials (NIT) conducted in Linxian, China. In the NIT General Population Trial, nine nutrients including Zn were studied. Zn dose was 22.5 mg/d. At the end of this trial, an endoscopic survey was carried out.[44] Other reports come from the NIT Dysplasia Trial. In the mentioned study, 3318 individuals who had been previously diagnosed with esophageal dysplasia by balloon cytology, received multivitamins and mineral supplements that included Zn, or placebo for 6 years. Three studies reported different outcomes from this trial.[46,47]

DISCUSSION

According to our knowledge, this systematic review is the first study evaluating the association between Zn biomarkers and EC. Nineteen studies were included in this review, and most of the observational studies reported an inverse association between Zn biomarkers and EC. This inverse association was observed in populations with different baseline Zn concentrations and in subjects from different countries. However, we found no single agent intervention study to summarize and the multi agent trials have produced conflicting results without clear evidence of benefit.

Table 1: **Observational studies of Zn biomarkers and esophageal cancer**[1]

Author	Year	Design	Country	Men among control %	Mean age of control y	Type of control subjects	Source of case subjects	Outcome	No of case subjects/ non case subjects	Zn assessment (technique)	Zn concentration: Case subjects	Zn concentration: Non case subjects	Unadjusted OR (95%CI)/ *p*	Adjusted OR (95%CI)/ *p*
O'Rorke[29]	2012	CCS	Ireland	83.3	63.6	General practitioner lists	Ireland case control study	EA incidence	137/221	Toenail (INAA)	70.7±21 µg/g	70.1±18.5 µg/g	0.87 (0.52-1.45)/ 0.55	0.86 (0.51-1.46)/ 0.56
Ray[30]	2012	CCS	South Africa	NR	NR	Volunteers from General population	Hospital	ESCC Prevalence	30/30	Hair (AAS)	0.20±0.11 ppm	0.39±0.10 ppm	NR, *p*<0.0001	NR
			India								0.54±0.21 ppm	0.64±0.23 ppm	NR, *p*=0.08	NR
Sun[31]	2011	CCO	China	69	58	Normal tissue from the same patient	Hospital	ESCC incidence	36/36	Tissue (AAS)	16.51±1.28 µg/g	20.44 ±1.55 µg/g	NR, P<0.01	NR
Dar[32]	2008	CCS	India	65	NR	NR	Institute of medical sciences	ESCC prevalence	55/55	Plasma (AAS)	86.8 µg/dl	96.1 µg/dl	NR, *p*<0.0001	NR
Nouri[33]	2008	CCS	Iran	43	NR	Hospital/ family	Hospital	ESCC incidence	20/80 (60+20)	Nail (AAS)	126.5 ±42 ppm	Sari= 173±111 Tehran= 251±213 Family= 175±131	NR, *p*<0.001	NR
Goyal[34]	2006	CCS	India	69	44	NR	NR	ESCC incidence	24/23	Serum (AAS)	75.20±5.57 µg/dl	87.17 ±6.43 µg/dl	NR, *p*<0.001	NR
Dursun[35]	2006	CCS	Turkey	50	50.2	NR	NR	NR	17/20	RBC SOD	1.87±0.10 U/mg Hb	1.67±0.16 U/mg Hb	NR, *p* < 0.001	NR
Rogers[37]	1993	CCS	USA	74	NR	Cancer registry	General population	EC incidence	73/434	Nail (NAA)	NR	NR	NR	1.7 (0.7-4.1), NR
Prasad[38]	1992	CCS	India	65	56.4	Hospital	Hospital	ESCC incidence	35/35	Plasma (AAS)	10.2±0.22 µmol/l	13.9±0.56 µmol/l	NR, *p*<0.001	NR
Mellow[36]	1983	CCS	USA	100	55	Hospital personnel	Hospital	ESCC incidence	17/10	Plasma (AAS)	65.7 ± 3.3 µg/dl	80.5±2.4 µg/dl	NR, *p*<0.01	NR
Abnet[39]	2005	Cohort	China	47	55 (49-59) median	Nested in cohort	Nested in cohort	ESCC incidence	60/72	Tissue (X-ray fluorescence)	44 (30-75) ng/cm^2	57 (47-108) g/cm^2	NR	HR=0.74 (0.56-0.97)/ 0..015

[1] AAS, Atomic Absorption Spectrometry; **INAA**, Instrumental Neutron Activation Analysis; **CCS**, Case-Control Study; **CCO**, Case Crossover Study; **NR**, Not Reported; **RBC SOD**, Red Blood Cell Super Oxide Dismutase; **ppm**, point per million; **Hb**, Hemoglobin

Table 2: Randomized trials of Zn and esophageal cancer

Author	Year	Country	Population	Men	Mean age	Zn form (dose mg)	Other vitamins or minerals combined with Zinc	No of subjects	Factorial design	Placebo controlled/ Double blind	Intervention period	Follow up After trial	Outcomes	Relative risk	Quality score[1]
Wang[40]	2013	China	Patients with dysplasia	44	54	Zn sulfate (45)	14 vitamins & 12 minerals/ daily	3318	No	Yes	6 y	20 y	Total mortality/ Total cancer mortality/ EC mortality	No effect	4
[2]Qiao[41]	2009	China	Residents in Linxian	45	52 at start	Zn oxide (22.5)	5000IU retinol palmitate/ daily	29584	Yes	Yes	5.25 y	10 y	Total mortality/ Total cancer mortality/ EC mortality	Increased total and stroke mortality	4
Taylor[42]	1995	China	Patients with dysplasia	44	54	Zn sulfate (45)	14 vitamins & 12 minerals/ daily	396	No	Yes	30 mo	0	Reversion to non-dysplasia	1.26 (1.06-1.46)/ p=0.005	4
											72 mo	0	Reversion to non-dysplasia	1.21 (1.02-1.40)/ p=0.02	
Zhang[43]	1995	China	Residents in Linxian/ Patients with dysplasia	45/ 44	52/54	Zn oxide (22.5) /Zn sulfate (45)	14 vitamins & 12 minerals/ daily	400/375	Yes	Yes	5.25 y/6 y	0	T cell response	No effect	4
Taylor[44]	1994	China	Rencun commune	50	48 at start	Zn oxide (22.5)	5000 IU retinol palmitate/ daily	391	Yes	Yes	5.25 y	0	Prevalence of esophageal cancer	OR=1.02 (0.36-2.91)	4
													Prevalence of esophageal dysplasia or cancer	OR=1.12 (0.57-2.20)	
Rao[45]	1994	China	Patients with dysplasia	42	57	Zn sulfate (45)	14 vitamins & 12 minerals/ daily	512	No	Yes	30 mo	0	Overall amount of proliferation	p>0.05	4
													Lower epithelial level	p>0.05	
Wahrendorf[46]	1988	China	Residents in Huixian	50	35-64	Zn (50) / weekly	50000 IU retinol, 200mg riboflavin/ weekly	610	No	Yes	13.5 mo	0	Prevalence of precancerous lesions	OR=0.78, p=0.05	3
Munoz[47]	1987	China	Residents in Huixian	50	35-64	Zn (50) / weekly	50000 IU retinol, 200mg riboflavin/ weekly	170	No	Yes	13.5 mo	0	Prevalence of micronuclei in esophageal cells	OR=0.61, p=0.04	3

[1] Quality score ranges from 0 (worst quality) to 5 (best quality), based on criteria by Jadad et al.
[2] The references[40,42,43,45] and [41,44] and [46,47] are different outcomes from the same study.

Most of the observational studies were case-control studies, which present more opportunities for bias than cohort studies. Thus the evidence for a protective effect of higher zinc status against EC is questionable. Observational study results are consistent with animal studies. In animal models, a Zn deficient diet causes a precancerous condition in esophagus[1] and enhances the effects of esophageal carcinogens (e.g., N -nitrosomethylbenzylamine)[6] by different mechanism.

Only one case-control study reported no association between a Zn biomarker (toenail concentration) and risk of EA. Two studies did not specify the histological type.[35,37] All other studies which found a significant association were carried out using ESCC cases. The risk factors of these two types are different.[19] This conclusion should be interpreted cautiously because only one EA was included in our study. This contradiction may be related to the geographic area, as well. This study was carried in Ireland while most ESCC studies were done with participants from different regions of Asia. A recent meta-analysis reported a significant association between Zn intake, estimated using FFQ, and the risk of digestive tract cancers in Asia, but not in European or American populations.[48] The authors concluded that the different source of zinc intake may explain the different results in geographic region subgroups.

Future well designed studies examining the association between Zn biomarkers and EC are warranted. Careful consideration of choice of biomarkers will be important. All biomarkers of Zn, such as hair, nails, urine, or plasma may reflect Zn exposure to some degree.[4,49,50] However, the interpretation of biomarkers is not simple because circulating Zn concentrations respond to conditions such as inflammation. Nails are susceptible to soil contamination. Contamination by coloring dyes and antidandruff shampoos may limit the suitability of hair. And all observational studies can be affected by confounding factors including socioeconomic status, smoking, or other EC risk factors which could cause the apparent inverse association observed between Zn biomarkers and EC.

In all reported trials, Zn was given combined with other vitamins or minerals. These interventions with supplements containing multiple nutrients do not allow evaluation of the effects of Zn alone. In addition, all reported trials were done in China. Baseline nutritional status of the populations may influence the results.

In the Linxian NIT trials, all studies were null, with the exception of one analysis which reported a positive effect of Zn on reversion to non-dysplasia after 30 and 72 month of starting trial. In the NIT General Population Trial, Zn was co-administered with retinol and there was no apparent effect on ESCC incidence or mortality. Two other trials in Huxian, China, assessed the effect of Zn in combination with retinol and riboflavin versus placebo; this combination was effective in reducing the prevalence of micronuclei in esophageal cells[47] and precancerous lesions.[46]

The discrepancy between observational studies and the intervention trials could be related to the dose of the intervention agent, the formula of the intervention agent, the age at which the intervention started, or the duration of the intervention. Observational studies may reflect long-term intake of nutrients, whereas trials, have relatively short intervention periods, while cancer has a long latency period. Moreover, different doses may lead to different results and subjects with high or low baseline status may react differently to the intervention.

Currently, observational studies of Zn biomarkers suggest that higher Zn status is associated with reduced risk of EC, but the evidence base is limited by the small number of studies and that many had weak study designs and small sample sizes. Well designed and larger cohort studies are needed before any conclusions can be drawn. Furthermore, current trial data does not suggest that supplements delivered in middle age are beneficial. The evidence base here is also limited by the lack of single agent Zn intervention trials and that most work has been conducted in a single population in China.

In conclusion, an inverse association between Zn concentrations and EC incidence was apparent in most of the reviewed observational studies, but the validity of such studies is uncertain. Randomized trials did not yield any evidence on the beneficence of Zn, but there are many limits to the current evidence base. Overall, the role of Zn in EC incidence is unclear and the benefits of Zn supplementation are not apparent. Yet the strong evidence from animal studies suggests that this hypothesis deserves further consideration.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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Appendix 1: **Quality criteria for observational studies on Zn and esophageal cancer**

	Case-control studies										Prospective cohort studies
Reference number	29	30	31	32	33	34	35	36	37	38	39
All observational studies											
Exposure was assessed at the individual level	√	√	√	√	√	√	√	√	√	√	√
Outcomes were based on objective tests or standard criteria in 90% of study participants	√	√	√	√	√	√	√	√	√	√	√
The authors presented internal comparisons within study participants	√		√	√	√		√	√	√		√
The authors controlled for potential confounding risk factors in addition to age	√			√	√		√	√	√	√	√
Prospective cohort studies											
Loss to follow-up was independent of exposure											√
The intensity of search of disease was independent of exposure status											√
Case-control studies											
Data were collected in a similar manner for all participants	√	√		√	√	√	√		√	√	
The same exclusion criteria were applied to all participants	√	√							√		
The selection process for Non cases was described	√	√			√				√	√	
The study was based on incident cases of disease	√					√		√	√	√	