

Original Article

Nail Molybdenum and Zinc Contents in Populations with Low and Moderate Incidence of Esophageal Cancer

Mohsen Nouri MD*, Hamid Chalian MD*, Atiyeh Bahman MD*, Hamid Mollahajian MD*,
 Mohammadamin Ahmadi-Faghieh MSc***, Hafez Fakheri MD†,
 Ahmadreza Soroush MD**

Background: The objective of our study was to compare the zinc and molybdenum contents of nails in two populations at high and low risk for esophageal cancer in northern Iran.

Methods: Four groups were studied including a control group in Tehran—a low-risk area (n=20); a group of patients with esophageal cancer from Sari—a moderate- risk region in northern Iran (n=20); a group of patients' family members (n=20); and a control group in Sari (n=40). Molybdenum and zinc levels were measured in their nail samples using flame atomic absorption spectrometry.

Results: Both molybdenum and Zinc were significantly ($P<0.01$) lower in the Sari control group (molybdenum: 0.472 ppm, zinc: 173.0 ppm) compared with the Tehran control group (molybdenum: 0.740 ppm, zinc: 251.5 ppm). These elements were lower in content in patients with esophageal cancer (molybdenum: 0.283 ppm, zinc: 126.5 ppm) compared with the Sari control group ($P<0.05$ for molybdenum and $P<0.001$ for zinc). Only zinc was significantly ($P<0.001$) lower in patients compared with their family members (molybdenum: 0.456 ppm, zinc: 175.5 ppm). No differences were found between the Sari control group and the patients' family members in any of the two elements.

Conclusion: Zinc and molybdenum levels are much lower in north of Iran—a moderate- risk area for esophageal cancer—compared with Tehran—a low-risk area. Based on these results, more investigations on the relations of trace elements with esophageal cancer in high-risk areas in northern Iran are warranted.

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Introduction

Several studies have suggested preventive roles for molybdenum (Mo) and zinc (Zn) in esophageal cancer (EC). A study on the rat model of nitrosobenzylmethylamine (NBMA)-induced esophagus tumor showed that

dietary Zn deficiency increases the incidence and shortens the lag time for esophageal tumor induction.¹ Another experimental study, in which various cereals and dietary staples were fed to inbred rats, showed that the number of animals which developed esophageal tumors after NBMA injection is significantly related to the dietary concentration of some minerals and vitamins. Wheat diets with various combinations of nicotinic acid, riboflavin, Zn, magnesium, Mo, and selenium significantly reduce the number of esophageal tumors.²

In addition to the experimental studies, several retrospective and prospective human population-based surveys have underlined the role of Zn and Mo deficiency as risk factors for development of EC. In one study carried out in China in which the

Authors' affiliations: *Jostar Research Group, Students' Scientific Research Center, **Department of Surgery, Shariati Hospital, Tehran University of Medical Sciences, ***Laboratory of Chemistry, Atomic Energy Organization of Iran, Tehran, †Department of Gastrointestinal Disease, Imam Khomeini Hospital, Sari University of Medical Sciences, Sari, Iran.

•Corresponding author and reprints: Ahmadreza Soroush MD PhD, Department of Surgery, Shariati Hospital, Tehran University of Medical Sciences, Kargar Shomali St., Tehran 14114, Iran.

Fax: +98-218-863-3039, E-mail: info@dr-soroush.com

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average of daily intake of Zn was estimated, EC mortality among a vegetarian population with low dietary Zn intake was 5.3 times higher than a vegetarian population with high dietary Zn intake.³

In one environmental study, epidemiologic data for EC in the Butterworth District, Transkei, revealed that Zn and Mo levels in soils tended to be substantially lower in the high-risk regions for EC which were on sedimentary strata compared with low-incidence zones in the study regions which were underlain by dolerite intrusions.⁴

To investigate food patterns, nutrient intakes, and seasonal variation, dietary surveys were conducted among the residents of Linxian, an area in the north-central China with some of the world's highest rates of EC. These surveys indicated that the nutrient intake in Linxian was inadequate for a number of vitamins and minerals including Zn.⁵ In a case-control study conducted in western Washington state, individuals who reported dietary intakes of Zn in the upper quartile were less likely to develop EC than were individuals with Zn intake in the lowest quartile. However, no significant differences in Zn concentrations in nail tissue was detected between subjects with EC and controls.⁶ In another study where postmenopausal women who completed a food frequency questionnaire, were followed for 16 years, after mutually adjusting Zn intake in a dose-response manner, the intake was shown to be inversely associated with the risk of upper digestive tract cancer.⁷ Using X-ray fluorescence spectroscopy to measure Zn concentration in single five-micron-thick sections from biopsy specimens from EC cases and control subjects, it was shown that the risk of developing EC was much lower for subjects in the highest quartile of esophageal tissue Zn concentration compared with those in the lowest quartile.⁸

Not all studies of vitamins, minerals, and EC have produced convincing results. Dysplasia Trial in Linxian was conducted among residents with cytologic evidence of esophageal dysplasia and found only modest, statistically nonsignificant protective effects on mortality in the mineral- and vitamin-supplemented group.⁹ In General Population Trial in Linxian, no statistically significant reductions in the prevalence of esophageal or gastric dysplasia or cancer were seen for any of the four vitamin/mineral combinations including vitamin C/Mo and retinol/Zn used by the patients who had previously been diagnosed with clinically-silent precancerous lesions and early

invasive cancers of the esophagus or stomach.¹⁰ Finally, no significant effects on mortality rates from all causes in a population with some of the world's highest rates of esophageal and stomach cancer were found for supplementation with retinol and Zn, riboflavin and niacin, or vitamin C and Mo in Nutrition Intervention Trials in Linxian.¹¹

According to the variable observed results reviewed above, it seems that there is a need to clarify the role of trace elements in EC. Furthermore, it is unknown that how applicable the results from China are to a geographically, environmentally, culturally, and ethnically different high-risk population such as what is found in northern Iran. Golestan Province in the north-east of Iran, along with Linxian Province in China, is among the highest rates of esophagus squamous cell carcinoma (ESCC) in the world. While several large-scale historical and interventional studies (some of which mentioned above) tested the effect of minerals, vitamins, and trace elements on ESCC in China, the necessity of performing such studies in Iran is felt. Although some studies in Iran have addressed the role of minerals and trace elements like Se in ESCC,¹² no studies have evaluated Zn and Mo in northern Iran which has a high incidence of ESCC. Surveys have shown that soil and foliage Mo content in north-eastern Iran is much lower than other regions such as north-western provinces.¹³ These findings, along with the previous investigations conducted in China, led us to hypothesize that Mo and Zn deficiency in Sari, the Capital of Mazandaran Province, might be associated with high incidence of EC.¹⁴

Materials and Methods

We studied four groups:

Group 1 (Tehran control): Twenty healthy people, accompanying patients attending Shariati Hospital in Tehran, aged 45 – 55 years, who lived for the last five years in Tehran and who had no personal or family history of any types of cancer.

Group 2 (Sari control): Forty healthy people, accompanying patients attending Imam Khomeini Hospital in Sari, aged 45 – 55 years, who lived for the last five years in Sari and who had no personal or family history of any types of cancer.

Group 3 (patient group): Twenty patients diagnosed with ESCC within the last two months and who have lived for the last five years in Sari.

Group 4 (patient's family group): Healthy first-

degree family members of the patients in group 3 who have lived for the last five years in the same home with patients and eaten the same diet.

Data sheets for each subject were completed providing information on age, sex, habits (drinking tea and alcohol, and smoking), and personal and family history of major diseases. All subjects in groups 1, 2, and 4 were visited by a physician who asked about cardinal symptoms of cancer (e.g., weight loss, dysphagia, changing bowel habits, etc.), and examined the subjects to determine their health status. This was done to reduce the probability of possible effects of chronic diseases on Zn and Mo contents.

Toe nails from each subject were clipped out by a nurse, packed in special containers, and sent for chemical analysis to the Chemical Department of Atomic Energy Organization of Iran. The samples were labeled with random numbers to blind the laboratory examiner.

Samples weighing 100 – 300 mg were cleaned with successive portions of acetone, alcohol, and distilled water and again, with acetone. Then, the samples were dried at 60°C and weighed again. For digestion, five milliliter of two N nitric acid (Merck, Germany), was added to the samples and left for 24 hours. Then, the samples were heated using a temperature-controlled digestion stand for a few hours until the solution was clear and the resulting solution was completely evaporated. Finally, the samples' volume was adjusted to five milliliter using distilled water. Air-acetylene flame atomic absorption spectroscopy and graphite furnace atomic absorption spectrometry (Varian Model 220-Spectra Atomic Absorption) were employed for measuring Zn and Mo, respectively. To standardize the measurements, standard solutions of Zn (Merck, Germany) and Mo (Sigma-Alderich, Germany) were utilized. Levels higher than 0.02 ppm of Zn and higher than 0.001 ppm of

Mo were detectable.

The data were analyzed by SPSS (version 13.0) utilizing Pearson's correlation test, Kruskal-Wallis (KW), Mann-Whitney U (MWU), and Wilcoxon rank tests.

Results

No significant sex differences in the content of Zn or Mo were found in any of the four groups. Only three cases reported alcohol use. Smoking was not significantly different among the groups (KW, $P>0.05$) while drinking tea was (KW, $P<0.05$). The Sari control group drank tea more frequently than the Tehran control group (MWU, $P<0.01$). Patients' tea consumption was significantly more than their family members' (MWU, $P<0.05$) and that of people in the Sari control group (MWU, $P<0.001$); yet, the latter two groups showed no significant differences.

There were significant differences among the four groups regarding subjects' age (KW, $P<0.001$). Though subjects of the Sari control group were significantly older than the patients' family members (MWU, $P<0.01$), neither Mo nor Zn showed significant differences between these two groups (KW, $P>0.05$). Also, by comparing Mo and Zn contents between the people younger and older than the median age in each group, no significant differences were detected (MWU, $P>0.05$). These findings, altogether, discount the role of age in confounding the results.

All samples had measurable concentrations of both Zn and Mo. Pearson's correlation test showed positive correlation between Zn and Mo contents ($P<0.01$, $r=0.328$). KW test showed highly significant differences in nail contents of both Mo ($P<0.01$) and Zn ($P<0.001$) among the groups (Table 1). Cases of the Tehran control group had significantly higher contents of Zn and Mo

Table 1. Distribution of age, sex, and nail zinc and molybdenum in the study sample.

Group	n	Median age (yr)	Male (%)	Median nail zinc (ppm*)(IQR [†])			Median nail molybdenum (ppm*)(IQR [†])		
				Male	Female	Total	Male	Female	Total
Sari control group	40	47.0	19 (47,5)	161.5 (116.0)	178.0 (119.7)	173.0 (111.7)	0.472 (0.382)	0.478 (0.194)	0.472 (0.307)
Tehran control group	20	53.0	10 (50)	221.0 (339.0)	251.5 (193.0)	251.5 (213.25)	0.603 (0.973)	0.906 (1.011)	0.740 (0.962)
Patient group	20	65.0	9 (45)	126.0 (24.0)	137.0 (72.0)	126.5 (42.25)	0.286 (0.591)	0.242 (0.437)	0.283 (0.346)
Patients' family group	20	37.5	6 (30)	136.0 (95.5)	192.0 (131.5)	175.5 (131.75)	0.429 (0.431)	0.458 (0.351)	0.456 (0.339)

*Part per million, [†]Interquartile range.

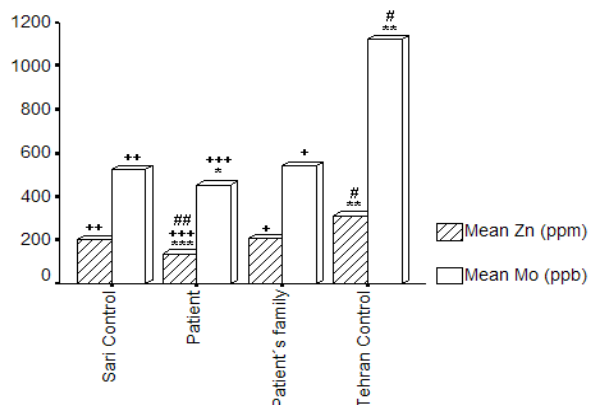


Figure 1. The mean concentration of zinc and molybdenum measured in different groups. Zinc levels are presented in part per million (ppm) while molybdenum's unit is part per billion (ppb). One, two, or three mark(s) represent significant differences where P value is less than 0.05, 0.01, or 0.001, respectively. *Compared with Sari control group; +Compared with Tehran control group; #Compared with patients' family group.

compared with the Sari control group ($P < 0.01$), patient group ($P < 0.001$), and patients' family group ($P < 0.05$) using MWU (Figure 1). Cases in the Sari control group had significantly higher Zn and Mo contents compared with the patient group (MWU, $P < 0.05$ for Mo and $P < 0.001$ for Zn). As mentioned earlier, neither Zn nor Mo showed statistically significant differences between the Sari control group and the patients' family group using MWU. However, both nonparametric Wilcoxon rank and MWU tests showed significant difference between the patient group and the patients' family group in Zn content ($P < 0.001$) but did not detect any statistically significant differences in Mo content.

Discussion

The results of this study are concordant with previous findings claiming that soil and foliage Mo content in the north-east of Iran are much lower compared with other regions. Zn and Mo contents in nail samples of people from Tehran were much higher than the people from Sari; yet, small simple sampling which was used in this study may be claimed to not be accurate enough to represent these contents in the whole population and sampling error cannot be ruled out. Nonetheless, this study—as a pilot study—was designed just to provide an overall impression about the Mo and Zn

contents among the above-mentioned groups—an aim accomplished. Comprehensive studies with samples representative for the general populations are needed to obtain a more distinct impression.

Zn and Mo contents were equal in the families who had a member diagnosed with ESCC with those families without a cancer patient but from the same city, Sari. This shows that decreased Mo and Zn contents are homogeneously present in all families residing in Sari, and not in certain families. If families with an ESCC member have lower Mo or Zn compared with other families in that area, some indigenous factors, say certain dietary habits or genetic traits exist among these families, may come into play and increase their susceptibility to cancer. However, these homogeneously reduced contents indicate a regional and widespread rather than a familial and limited cause. As Mo deficiency has been documented in the foliage and soil of that region, more studies to investigate Mo and Zn contents of the drinking water and food resources used by the people of that area may be associated with some promising results.

Patients with ESCC had lower contents of Zn, but not Mo, compared with their family members. This finding can be explained by different hypotheses among which we review three here:

1) ESCC directly decreases Zn levels in the body by increasing metabolism rate or take-up of the Zn by the tumoral cells; 2) as dysphagia raises as a symptom of the disease, this may affect nutrients intake including Zn; and 3) these patients had some severe Zn deficiency (e.g., due to genetic or nutritional reasons) before being affected by ESCC and this deficiency was the likely cause of their cancer. On the other hand, Mo contents did not change in ESCC patients compared with their family. This can also be discussed by different hypotheses: 1) this type of tumor does not affect Mo's metabolism as it does Zn's; 2) our bodies have more Mo reserves which help us keep the Mo level in spite of the increased metabolic state and decreased food intake; and 3) water provides enough Mo that eliminates the necessity of using Mo reserves. It goes without saying that liquid intake is affected lately in the course of the disease; so, water intake is affected only in the late stages, if at all.

In conclusion, we found that people of the north of Iran, which has a high incidence of ESCC, have lower levels of Mo and Zn than the Tehran controls. More comprehensive and meticulously-

designed studies are required to research the food resources used in that region which lacks these elements and to evaluate any possible effects of Mo and Zn supplements on the incidence of ESCC.

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