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— SHORT COMMUNICATION —

Spectroscopic studies on molybdenum and zinc levels in fingernails of patients with esophagus cancer

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Molybdenum (Mo) and zinc (Zn) are well-known bio-essential trace elements, and their anti-carcinogenic activity has been researched. In this study, Mo and Zn concentrations were investigated in fingernails of 40 patients (20 males, 20 females, mean age 56.0 ± 13.4 years) with esophagus cancer and 46 healthy subjects (23 males, 23 females, mean age 53.6 ± 12.5 years) using atomic absorption spectrometry (AAS). The average concentrations of Mo and Zn were 0.45 ± 0.37 and 136.8 ± 32.5 mg l^{-1}, respectively in the patients and 1.10 ± 1.09 and 310.9 ± 151.8 mg l^{-1} in the healthy population. Mo and Zn levels were found to be decreased in the patients affected by esophagus cancer compared with the control group ($p < 0.01$ and $p < 0.0001$, respectively). The content of Mo in the human nail is related to the Mo level in the environment. The soils and sediments greatly affect Mo concentrations in human. Increased dietary intake of Mo and Zn may decrease the risk of esophagus cancer.

Key words: molybdenum, zinc, fingernails, esophagus cancer.

INTRODUCTION

Esophagus cancer is a malignancy of the esophagus. This cancer remains a devastating disease, because it is usually not detected until it has progressed to an advanced incurable stage. Esophageal tumors usually lead to dysphagia, pain and other symptoms, and are diagnosed with biopsy. Small and localized tumors are treated with surgery while advanced ones with chemotherapy, radiotherapy or combinations of these. Prognosis depends on the extent of the disease and other medical problems, but it is fairly poor.

Trace elements in human body tissues are particularly interesting as to their role in the body, from the point of view of their relationship with diseases. They exist in very low concentrations in the body, consisting of 0.01% of the total body weight (Vural *et al.*, 2000). Trace elements have been shown to influence a number of biochemical and physiological processes. In the case of insufficient intake of trace elements with the diet, besides various insignificant pathologic findings, other life-threatening pathologic results may also appear.

As early as in 1953, molybdenum (Mo) was recognized to be an essential trace element for many species including man (Morrison & Risby, 1979). It is a component of many enzymes responsible for the initial stages of nitrogen, carbon and sulfur metabolism of plants, animals and man, and participates in a large number of enzymatic reactions (Coughlan, 1980; Burgmayers & Stiefel, 1985).

Mo has also important physiological functions in the human body. This metal is found in most foods, with legumes, dairy products, and meats being the richest sources. Mo is considered essential because it is part of a complex called Mo-cofactor that is required for three mammalian enzymes, i.e. xanthine oxidase (XO), aldehyde oxidase (AO) and sulfite oxidase (SO). XO participates in the metabolism of urine, AO catalyzes the conversion of aldehydes to acids and SO is

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involved in the metabolism of sulfur-containing amino acids (Sardesai, 1993). The content of Mo in the human body may be related to the Mo level in the environment. Low Mo levels in soil, plants, drinking water, food and human tissues may be responsible for high mortality due to esophagus cancer. It is useful, therefore, to provide people with a proper supplement of Mo or use Mo-containing fertilizer to prevent carcinoma development (especially esophagus cancer).

Dietary Mo deficiency has never been observed in healthy people. The biochemical signs of Mo deficiency include low plasma uric acid levels, decreased urinary excretion of uric acid and sulfate, and increased urinary excretion of sulfite. Thus, the patient is diagnosed by defects in uric acid production and sulfur amino acid metabolism. The recommended dietary allowance (RDA) for Mo was revised recently (January 2001). It was based on the results of nutritional balance studies conducted in eight, healthy young men under controlled laboratory conditions (Food and Nutrition Board, 2001).

Zinc (Zn) is the most ubiquitous of all trace elements involved in human metabolism. More than 100 specific enzymes require Zn for their catalytic function. In addition, Zn acts as antioxidant. Zn is mainly required for DNA synthesis, cell division and protein synthesis. It directly stimulates DNA synthesis by altering the binding of F and F_3 histones to DNA so as to affect RNA synthesis. It has been hypothesized that Zn could be operating at several levels influencing lymphocyte monoclonal proliferation (Goyal *et al*., 2006). The inhibitory effects of Zn and Mo on carcinogenesis have been reported in epidemiological studies (Abu Baker *et al*., 2005; Lu *et al*., 2006).

This study has been conducted on subjects of the Sari city (Iran). Sari is a small region in northern Iran, where the incidence of esophagus cancer is very high (six times higher than the average in Iran). The aim of this study was to investigate the relationship between esophagus cancer and Mo and Zn levels in human and the environment.

It must be emphasized that studies on Mo metabolism in man have often be hampered by the lack of sufficiently sensitive analytical methods for monitoring Mo in human tissues and body fluids. For this reason, in many cases it is difficult to determine whether the symptoms attributed to Mo deficiency or excess are due to biological variations or simply to experimental error. A better understanding of the role of Mo in human nutrition depends on improving of the sensitivity and accuracy of the analytical methods involved. Only then, the biological function of Mo as an essential trace nutrient will be fully understood. In the present study, we applied graphite furnace atomic absorption spectrometry (GFAAS) for determining Mo in fingernails. GFAAS is the most readily available technique for the determination of very low Mo contents in biological samples with its short measuring time, the high accuracy and the low sample consumption.

MATERIALS AND METHODS

Patients and controls

The study involved two groups of subjects. Group I (esophagus cancer) comprised 40 patients with esophagus cancer. There were 20 males and 20 females, aged 30 to 72 years. Group Π (controls) was composed of 46 healthy subjects, 23 males and 23 females, aged from 28 to 69 years, and recruited from the same area as the patients (Sari city). Zn and Mo concentrations in fingernails were measured during the study and were compared with the control group.

Sample collection and treatment

Fingernails were clipped with nail clippers or stainless steel scissors and were sent to the laboratory in envelopes marked with a code number, date of birth and sex. All the nail clippings obtained were free of gross contamination such as nail varnish or polish. The nails were scraped free of excessive dirt with a stainless steel spatula. Then, they were soaked in a cleaning solution (Contrad 70, International Reagents Corporation, Kobe, Japan), washed three times for 15 min in an ultrasonic bath, rinsed with distilled water and allowed to dry at 45°C for 24 hrs (Karita *et al*., 2001). After drying, approximately 0.1 g of nails from each sample was weighed and transferred into polytetrafluoroethylene (PTFE) vessels. Then a 3:1 mixture of HNO_3 and H_2O_2 (both of suprapure grade, Merck, Germany) was added and digested at 120°C for 2 hrs.

Determination of Mo and Zn in nails

Zn level in fingernails was determined by flame atomic absorption spectrometry (FAAS) using an AA220 (Varian, Australia) atomic absorption spectrometer. The digested samples were diluted (1:9) with deionized water. The quantification of Mo was performed by graphite furnace AAS (AA220, GTA 100) using pyrolytically coated graphite tubes and deuterium

TABLE 1. Optimized instrument parameters and working conditions for elemental analysis in human nail

A. Instrument settings for determination of molybdenum in human nail by GFAAS

Calibration mode	Measurement mode	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Sample volume (μl)	Modifier volume (μl)
Concentration	Peak area	313.3	0.5	7	10	5

B. Furnace optimized parameters for analysis of molybdenum in human nail by GFAAS

Element	Step	Temperature (°C)	Time (sec)	Argon flow-rate ($l\ min^{-1}$)
	Drying	85	5.0	3
	Pre last drying	95	40.0	3
	Post last drying	120	10.0	3
	Ashing	1100	5.0	3
Mo	Ashing	1100	1.0	3
	Gas stop	1100	2.0	0
	Ramp stop	2700	1.3	0
	Atomization	2700	2.0	0
	Tube clean	2700	2.0	3

C. Instrument settings for determination of zinc in human nail by FAAS

Calibration mode	Measurement mode	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Fuel gas	Oxidant gas
Concentration	Integration	213.9	1	5	Acetylene	Air

background correction (Marcezenko & Lobinski, 1991) after a further 1:4 dilution of the digests with deionized water. The matrix modifier consisted of 0.004 mol dm^{-3} strontium nitrate and 0.004 mol dm^{-3} nickel nitrate (Matsusaki *et al*., 1999) for determination of Mo in fingernails. The optimized instrumental conditions for determination of Mo and Zn are shown in Table 1. The accuracy of the procedures was checked with standard reference material (BCR-CRM 397).

Determination of Mo in soil

To study the relationship between Mo concentration in soil and esophagus cancer, soil samples were collected in different areas of Sari city. The samples were dissolved in a mixture of perchloric and hydrofluoric acids. Dissolution was followed by the development of thiocyanate complex, which was then extracted into methyl isobutyl ketone (Kim *et al*. 1974). Mo concentrations were determined using an atomic absorption spectrometer equipped with a graphite furnace (Khan *et al*., 1979). Standard calibration curves were prepared, and all samples and standards were measured in triplicate.

Statistics

Statistical evaluation was carried out by using the SPSS 11.5 for Windows. Summary statistics (n, mean, standard deviation, correlation coefficient) were calculated. Values were compared using one-way analysis of variance (ANOVA), also taking into account sex as a grouping variable. Correlation analysis between metal concentrations in nails was performed using the Pearson product-moment correlation coefficient. All results were expressed as mean values ± s.d. Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Mean nail Mo and Zn values of patients and controls with p values are given in Table 2. The mean concentrations of Mo and Zn were found to be significantly lower ($F = 5.4, p < 0.01$ for Mo and $F = 13.9, p < 0.0001$ for Zn) in patients (group I) compared with controls

TABLE 2. Molybdenum (Mo) and zinc (Zn) concentrations (mean ± s.d.) in nails from patients with esophagus cancer and healthy subjects

Element	Group I: esophagus cancer (n=40)			Group II: controls (n=46)			*p*		
	F+M	F	M	F+M	F	M	F+M	F	M
Mo (mg l^{-1})	0.45±0.37	0.39±0.28	0.53±0.47	1.10±1.09	1.33±1.30	0.82±0.69	< 0.01	< 0.05	0.32
Zn (mg l^{-1})	136.80±32.50	146.00±40.80	125.40±12.60	310.90±151.80	299.20±125.60	327.00±190.30	< 0.0001	< 0.0001	< 0.01

Values represent the levels of molybdenum and zinc for each group, considering the whole population (F+M), the females (F) and the males (M)

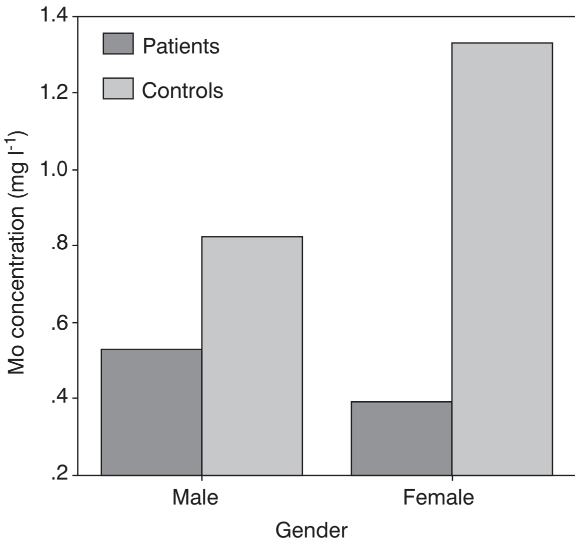

FIG. 1. Nail molybdenum levels (mg l^{-1}) in patients with esophagus cancer (n = 40) and controls (n = 46) in males and females.

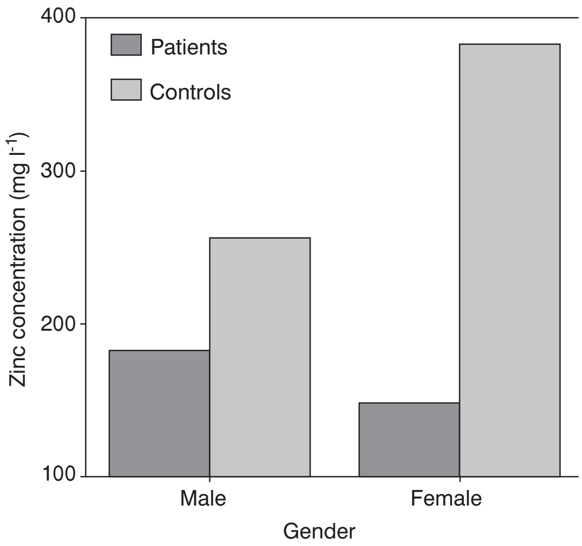

FIG. 2. Nail zinc levels (mg l^{-1}) in patients with esophagus cancer (n = 40) and controls (n = 46) in males and females.

(group Π), when both sexes were considered together (Figs 1, 2). However, when the two sexes were considered separately, no significant difference could be observed in Mo level between both groups for males ($F = 1.1$, $p = 0.32$).

We developed a reliable method for direct determination of Zn and Mo in fingernails in clinical practice. The nails are easy to sample and store and could be used to monitor changes in the level of incorporation of specific elements as a result of the disease status.

FAAS is a rapid and common laboratory technique capable of routine Zn analysis of nail samples. The experimental parameters which are known to affect the analytical signal in FAAS are wavelength, slit width, lamp current, burner position and flame composition. For optimization purposes, a standard solution corresponding to the linear working range of each element was used. The best set of parameters was considered the one providing the highest accuracy based on recovery studies and analysis on certified standard reference material (BCR-CRM 397). That was 98.4% for Zn, with lowest relative standard deviation (RSD) of 1.1%.

The whole procedure for determination of Mo in biological samples should always be carefully evaluated with respect to its lower concentration in these samples. GFAAS was applied for monitoring Mo in biological samples. A serious problem in the determination of Mo by GFAAS is carbide formation (Hoening *et al*., 1986). Carbides formed on the tube surface enhance memory effects resulting in increased and variable blanks and loss of sensitivity. Also, carbon deposits from undigested biological samples may magnify the above effects even further. For overcoming this problem, we used pyrolytically coated graphite tubes in monitoring Mo. The accuracy, tested on BCR-CRM 397, was 95.8% (RSD = 3.4%).

The values of Zn and Mo found in nails in the present study do not deviate from those previously reported (Iyengar *et al*., 1978; Parker, 1983). Quality control data were in a statistically acceptable range.

The findings of the present study indicate a strong association of Mo and Zn with esophagus cancer. In esophagus cancer, there are significantly lower levels of Mo and Zn in nails, when compared with the controls. The Pearson correlation coefficient between Mo and Zn was higher in patients than in healthy subjects, but statistically not significant ($r = 0.160$, $p = 0.076$ and $r = 0.067$, $p = 0.054$, respectively).

Mo and Zn are important microelements which not only regulate the physiological functions of various organs, but are also associated with the development of pathological changes in these organs. Adequate Mo minimizes the presence of nitrites and nitrates in plant tissues. In regions in which the soil is deficient in Mo and Zn, plants are impaired in their ability to metabolize nitrates to amino acids. This impairment permits potentially toxic nitrogen compounds to accumulate within plants that enter the human food supply. Nitrosamines and other nitrosyl compounds are found in pickled vegetables, smoked meats and drinking water of certain geographic regions where the incidence of esophageal squamous cell carcinoma is high.

The incidence of esophagus cancer is very high in Sari (Iran). The soil in this region contains low amounts of Mo and other mineral elements. The average concentration of Mo is 2.0 mg l^{-1} in the sediments and 2.5 mg l^{-1} in the soils (Kim *et al*., 1974), whereas the mean level of this element is 1.3 mg l^{-1} in the soils of Sari. Thus, when Mo content in the soil is low, plants preferentially convert nitrates to nitrosamines instead of using nitrate to synthesize amino acids. This results in an increased nitrosamine exposure for those who consume the plants. Adding Mo to the soil in the form of ammonium molybdenate may contribute to decreasing the risk of esophagus cancer by limiting nitrosamine exposure.

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