



## The Nail as a Biomonitor of Trace Element Status in Golestan Cohort Study

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### ABSTRACT

#### BACKGROUND

In the Golestan Cohort Study that was launched to investigate the causes of esophageal cancer, a complete biospecimen bank was established for storage of collected blood, urine, hair, and nail samples. The objective of this study was to evaluate the feasibility of nail samples as a biomarker of selected trace elements status.

#### METHODS

Thirty toenail samples were selected randomly from the participants of Golestan Cohort Study (GCS). The samples were cleaned and analyzed for selenium, mercury, chromium, iron, zinc, and scandium by instrumental neutron activation analysis at the University of Missouri Research Reactor Center. Pearson correlation coefficients were computed for selected trace elements concentration versus scandium concentration to assess terrestrial contamination.

#### RESULTS

The selenium, zinc, and mercury were not correlated with scandium, suggesting they were free from substantial contamination. The high correlations of scandium with iron and chromium suggest that the iron and chromium levels may be compromised by terrestrial contamination. The coefficients of variation for duplicate samples for selenium and zinc were 2.6% and 7.2%, respectively.

#### CONCLUSION

The nail samples from Golestan Cohort Study appears to be useable as a biomarker of selenium, zinc, and mercury and could be considered for use in future studies.

#### KEYWORDS

Minerals; Biomarker Validation; Toe nail; Selenium; Zinc; Golestan Cohort Study

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#### INTRODUCTION

Golestan Province in northeast Iran is an area with high incidence of esophageal cancer with age-adjusted rates reported to be over 100 per 100,000 per year in 1970,<sup>1</sup> although recent reports suggest that these rates have declined as the socioeconomic status of the population has improved.<sup>2</sup> To investigate the etiology of this disease, the Golestan Cohort Study was launched in January 2004 by the Digestive Diseases

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Research Institute (DDRI) of Tehran University of Medical Sciences, the US National Cancer Institute (NCI), and the International Agency for Research on Cancer (IARC).<sup>3</sup>

Minerals have been reported as modifiers of risk of esophageal cancer in animal studies.<sup>4,5</sup> Zinc (Zn), and selenium (Se) are believed to have both antioxidant and proapoptotic properties.<sup>6,7</sup> However, exposures to some trace elements, such as mercury (Hg) and chromium (Cr) have been identified as potential human carcinogen.<sup>8</sup> Prospective studies have shown that zinc has protective effect against esophageal cancer.<sup>9,10</sup> Selenium is associated with a reduced risk of esophageal cancer in some studies.<sup>11,12</sup> There are conflicting data regarding the association between Iron (Fe) and risk of esophageal cancer.<sup>13,14</sup>

Estimation of average dietary mineral intake only by food frequency questionnaires and using available food composition data is not accurate. One reason for the inaccuracy is that the bioavailability of minerals is altered by phytate and other substances in food.<sup>15</sup> Moreover, some physiological factors such as age and genotype may affect some minerals absorption.<sup>16</sup> Finally, the variability of some trace minerals in foods is highly variable. Therefore, studies of minerals status may benefit from the use of biomarkers of minerals rather than estimating the intake of minerals through questionnaires.<sup>17,18</sup> In the Golestan study a biospecimen bank has been established for blood, urine, hair, and nail samples to be used in cross sectional or nested case control studies.<sup>3</sup> The choice of using blood, urine, tissue, hair, and nail as the biomonitor of an element depends on the research hypothesis, the availability of the biomonitor, the metabolic deposition of the element into the biomonitor, and the risk of contamination. Serum and urine reflect recent intake.<sup>19</sup> Nail samples have been used in many cohort studies as a biomarker of intake of certain dietary components including some trace elements that readily bind to the fibrous proteins of keratin in nails. Moreover, the slow growth of nails allows integration of exposure over a longer time period. Several previous studies have shown that nails can be a reliable biomarker for trace element status, especially reflecting past year exposure.<sup>20</sup> Hair and nail have been widely used to moni-

tor exposure to Se, Hg, and Arsenic (As). However, nails are susceptible to soil contamination, especially in farmers. Consequently, it is important to consider the feasibility of these biosamples in pilot studies before evaluating the intake-disease associations in large studies. In this pilot study, we examined the feasibility of nail samples of Golestan Cohort Study as a biomarker for estimation of mineral intake.

## MATERIALS AND METHODS

During the baseline examination, subjects were asked to cut their toenails. Nail specimens were placed in labeled plastic bags and stored at room temperature. A pilot study was designed to assess whether contamination or low sample mass would confound the measurement of elements in the toenail samples. We randomly selected 30 nail samples from the participants of Golestan Cohort Study (GCS) who had died in accidents during the first 7 years of follow-up. The samples were analyzed for Se, Hg, Cr, Fe, Zn, scandium (Sc), and aluminum (Al) activity by instrumental neutron activation analysis (INAA) at the University of Missouri Research Reactor center (MURR).<sup>21</sup> The Al activity cannot be reported as a concentration because it is produced by neutron reactions with three isotopes; <sup>28</sup>Al, <sup>28</sup>Silicon (Si), and <sup>31</sup>Phosphorous (P). The element P is present in the nail at 0.1 wt%. To our knowledge, the elements Sc, Al, and Si are not biologically active but they are ubiquitous in the environment. It is therefore hypothesized that Sc and Al activity monitor environmental contamination that remains after the cleaning procedure.

Prior to analysis, the samples were prepared by removal of any nail polish using acetone and then immersion in 10% (v/v) nitric acid and sonicated for 10 minutes. After the acid wash the nail samples were rinsed, immersed in 18 MΩ-cm water and sonicated for 10 minutes and finally rinsed with 18.2 MΩ-cm water. After the cleaning procedure the samples were freeze dried and a dry weight was recorded using an analytical balance. Duplicate samples were prepared when the sample mass was large by tearing each nail sample into two samples.

The samples in this pilot study were analyzed

twice by INAA. The first analysis was a short INAA procedure for measurement of Se concentration and  $^{28}\text{Al}$  activity. The toenail samples were packaged in polyethylene vials and irradiated in a neutron flux of  $5.0 \times 10^{13} \text{ n/cm}^2/\text{s}$  for 7 seconds. After a decay time of 15 seconds, the samples were counted for 30 seconds using a high purity germanium detector.<sup>22</sup> The samples were co-analyzed with National Institute of Standards and Technology (NIST) Standardized Reference Material 1577 bovine liver. The second measurement was a long INAA procedure for Hg, Fe, Cr, Zn, and Sc. The samples are weighed in quartz vials and irradiated for 40 hours at a flux of  $6 \times 10^{13} \text{ n/cm}^2/\text{s}$ . After irradiation the samples were decayed for 5–15 days and counted for 2 hours each using a high purity germanium detector with an automated sample changer. A dead time correction was done using a live time correction. The samples were co-analyzed with the quality controls NIST Standardized Reference Material 1577 Bovine Liver, and NIST Standardized Reference Material 1571 Orchard Leaves.

The data were analyzed using STATA software (version 12, STATA Corp, College Station, TX, USA). Pearson correlation coefficients were computed for selected trace elements concentration versus Sc concentration. A P value less than 0.05 was considered as statistically significant.

## RESULTS

The average mass of samples was  $49 \pm 1 \text{ mg}$ . Prior to cleaning 24 of the 30 samples were noted as dirty. The Se, Hg, Fe, Cr, Sc, and Zn measured in the quality control materials are reported in table 1. The measured values are in agreement with the certified values.

The coefficient of variation (CV) for duplicate samples for Se was 2.6% and for Zn was 7.2%. Mean concentrations of selected minerals are shown in table 2.

In the data set the Sc and Al activity were highly correlated. As discussed, both Si and Al contribute to the measured Al activity. The Se, Zn, and Hg were not correlated with Sc and Al. However, Sc was highly correlated with Fe and Cr (table 3).

## DISCUSSION

Our results show that nail samples collected in Golestan Cohort study, are adequate and suitable as a biomarker of Se, Zn, and Hg, and could be analyzed for evaluating exposure–disease association.

The concentrations of Se, Zn, and Hg were not correlated with Sc or Al activity in our study. It has been suggested that the elements Sc, Al, and Si could be monitors of external contamination. Furthermore, Sc cannot be taken up by organisms actively; thus it is considered as an appropriate monitor of contamination.<sup>23</sup> We assume that Sc levels measured in the nail samples are due to the suspended particles attached. A high correlation of this element with a selected element suggests the presence of terrestrial contamination. Lack of a correlation with the potential terrestrial monitors Al and Sc and the normal or low values of Se, Hg, and Zn relative to other populations suggest that significant environmental contamination has not confounded the levels of these elements in the toenails.

The mean concentrations of selected minerals in our study were consistent with other studies. In another study in Iran, the concentration of Zn in nail in control group, was  $81.6 \mu\text{g/g}$  and was similar to our results.<sup>24</sup> In two healthy populations toenail Zn has been reported to range from 27 to  $240 \mu\text{g/g}$ .<sup>21,25</sup> The toenail level of Se in our study ( $1.12 \mu\text{g/g}$ ) is similar to that reported for a subset of the health professionals follow-up study ( $0.66$  to  $1.14 \mu\text{g/g}$ ).<sup>22</sup> We hypothesize that the Golestan province is not a selenium deficient region, because in an ecological study that was done in this province, the median for serum selenium was  $155$  ( $141$ – $173$ )  $\mu\text{g/L}$  in 100 healthy adults and all the subjects had a serum selenium more than  $90 \mu\text{g/L}$ .<sup>26</sup> These consistencies confirm that nail samples collected in Golestan Cohort Study, are suitable as a biomarker of Se and Zn.

However, the Hg values reported in table 2 are low in comparison with toenail Hg reported by Mazzaffarian and colleagues.<sup>27</sup> This finding could be due to low fish consumption in Iran.

This study has some limitations. The correlation coefficient may be affected by confounding variables. Since this was a pilot study, we used 30 samples and

**Table 1:** Concentrations of measured elements in quality control data. The error is reported as 1 standard deviation of the average value.

NIST SRM 1577		NIST SRM 1574		NCS DC 73347	
Measured µg/g	Certified µg/g	Measured µg/g	Certified µg/g	Measured µg/g	Certified µg/g
Hg	0.024±0.02	0.016±0.02	0.132±0.014	0.155±0.015	0.46±0.01
Cr			2.4±0.1	2.6±0.3	
Fe	263±4	268±8			
Zn	137±2	130±13	31±11	25±3	195±1
Sc			6.1×10-2± 2×10-3	6.3×10-2 ± 1.4×10-2	
Se	1.13±0.04	1.1±0.1	0.072±0.02	0.08±0.02	0.68±0.01
					0.60±0.04

**Table 2:** Mean concentrations of selected minerals in nail samples in pilot study of Golestan Cohort Study

Elements (µg/g)	Mean	Std. Error	95% Confidence Interval
Se	1.12	0.10	1.33
Hg	0.044	0.009	0.064
Cr	1.13	0.25	1.64
Fe	250	55	362
Zn	87.4	3.7	94.9
Sc	0.075	0.016	0.109

**Table 3:** Correlation between selected trace elements in nail samples in pilot study of Golestan Cohort Study

	Mass, g	Se	Hg	Cr	Fe	Zn	Sc	Al
Mass, g	1							
Se	-0.05	1						
Hg	-0.05	0.15	1					
Cr	0.23	-0.01	-0.25	1				
Fe	0.21	0.01	-0.34	0.84**	1			
Zn	0.24	0.36*	0.20	0.00	-0.05	1		
Sc	0.21	-0.02	-0.37*	0.82**	0.99**	-0.05	1	
Al	0.22	-0.00	-0.38*	0.80**	0.98**	-0.05	0.99**	1

\* $p < 0.05$ , \*\* $p < 0.001$

we could not do sensitivity analysis on different age or sex groups. However, such studies are needed before analysis of large samples in cohort studies.

In conclusion, the nail samples from Golestan Cohort Study appears to be a reliable biomarker of the Se, Hg, and Zn and should, therefore, be considered for use in future studies. However for the Fe and Cr levels, they may be compromised by terrestrial contamination.

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#### CONFLICT OF INTEREST

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

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