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Toenail selenium level among healthy residents of two Polish Districts

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The goal of this study was to evaluate the selenium mass fraction in toenail clippings taken from random inhabitants living in various areas of the Pomeranian (Northern Poland) and Lubuskie (Western Poland) Districts. Toenail clippings were analyzed by instrumental neutron activation analysis (INAA) giving means of 0.57±0.10 and 0.60±0.16 $mg \cdot kg^{-1}$ for the two areas, respectively, but the difference was statistically not significant. In additional, it was found that gender, age, body mass index (BMI), smoking, and selenium supplementation are factors with apparent effects to the selenium levels in toenail clippings.

Introduction

Selenium, as a component of selenoproteins, is involved in many structural and enzymatic processes. It is well recognized that selenium plays an important role in the thyroid hormone metabolism, maintaining the healthy immune system, and preventing the alteration in cells.[1,2] Due to its presence in glutathione peroxidase (GSH-Px) and other different mammalian enzymes, selenium possess the ability to prevent against oxidative stress and to affect DNA methylation, inflammation, and apoptosis.[3–6] Many publications provide important overviews of selenium metabolism and the function of identified selenocompounds.[7–12]

It is generally recognized that insufficient selenium daily nutritional intake may result in etiology of disease processes or, in some cases, in the intensification of an existing disease. Numerous epidemiological studies have demonstrated an inverse relation between low Se intake and the development of so-called civilization diseases, among others, some types of cancer.[13–17] Considering the possible risks associated with suboptimal selenium intake, the monitoring of Se status is relevant in order to anticipate on intervention actions. Over the past decades a great number of studies has been carried out on the evaluation of human biomarkers for the Se status and its relation to the daily intake. Depending upon the type of biological material to be used, it is possible to obtain short- and long-term information about selenium status. Urine and serum (short-term status), blood (medium-term status), as well as nail clippings, and hair samples have all been considered for assessment as indicator for the selenium status.[18–26] Nevertheless, compared to other biological samples, nail clippings have many advantages as indicators. Their collection is noninvasive and samples can easily be obtained by an unskilled worker. Furthermore, toenail selenium concentrations are reasonably stable indicators of exposure over the past 6–12 month period,[27] whereas urine and blood selenium concentrations represent merely recent intake.

So far, very few studies have been undertaken to investigate the selenium status in Poland. The majority of research on the Polish population revealed low selenium level in investigated biomaterials.[28,29] According to findings presented by Polish researchers, the relatively low levels of this element in human organism may result from the deficiency of selenium in the diet. Up to now, the assessment of selenium status in Poland was based on Se determination in body fluids and tissues samples like. whole blood,[30] serum,[31] plasma,[32] milk,[33] renal cortex, liver, and hair.[34] To our knowledge, none of the studies performed to date have examined the selenium levels in nail clippings of the Polish population. Therefore, we decided to use toenail clippings as an indicator for the selenium status in adult residents of Poland. Our study was carried out on samples taken from inhabitants of Pomeranian and Lubuskie Districts. The hypothesis is that the Se status in these regions may be different because of the variations of its content in soil and plants, as well as by differences in nutritional habits.

Experimental

Study populations

The study was conducted in two periods: between March and September 2006 and from February to May 2008 among residents of the two Polish districts. Toenail clippings were taken from 177 randomly selected inhabitants of Pomeranian District and 159 subjects from Lubuskie District. All participants were instructed to cut the free edges of toenails, place them in an envelope and return them to a designated institution. Moreover, they were requested to complete a questionnaire for information on, e.g., demographic background, anthropometric measures, alcohol consumption, smoking habits and dietary supplements

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use. The characteristics of the studied population are presented in Table 1. Due to apparent unreliable information concerning alcohol intake, this information was not used as a potential factor influencing the selenium concentration in toenail specimens.

Sample preparation and analysis

The toenail clippings samples from all 10 toes were using stainless steel clippers. The nails scratched with a quartz knife to remove the surface contamination, and successively cleaned by acetone and deionized water. The rinsing process were done in an ultrasonic bath for 15 minutes each time, in order to improve cleaning efficiency. The samples were then freeze-dried to eliminate any humidity variations between runs. After weighted into pre-cleaned polyethylene vials, toenails were sent to the Reactor Institute Delft (the Netherlands) for the Se determination by instrumental neutron activation analysis (INAA).

The samples were irradiated for 17 s at a thermal neutron fluence rate of $4.1 \cdot 10^{16}$ $m^{-2} \cdot s^{-1}$, allowed to decay and counted for 17 s using a high resolution γ-ray spectrometer equipped with a digital spectrometer with dead-time correction option. The cyclus was repeated 7 times, and all spectra were accumulated to a final spectrum for analysis and interpretation. The associated software is optimized for accounting the decay of the short half-life ^{77m}Se amongst the various cycles in this pseudo cyclic NAA procedure.

Quality assurance

The trueness of the method was checked by running twenty replicates of the certified reference materials from National Institute of Standards and Technology NBS-1577b (bovine liver). The mean selenium concentration obtained for the reference material (0.764±0.030 $mg \cdot kg^{-1}$, 1 SD) agreed with certified value (0.7300±0.0030 $mg \cdot kg^{-1}$) at the 95% confidence level.

Statistical analysis

Statistical analyses were performed with the Statistica software, version 8.0. The selenium was not normally distributed; the non-parametric tests (Manna Whitney U test and Kruskal Wallis test) were therefore applied to assess any relationships between concentrations of selenium found in the toenail samples of the Districts' residents of both genders and different variables. Five (5) results were excluded from the final dataset because of extremely high toenail selenium concentrations (higher than 1.1 $mg \cdot kg^{-1}$), which might be caused by a low weight of samples. The possible relations between age and body mass index (BMI) of participants and toenail selenium level was based on correlations coefficients (Spearman rank). A probability value of $p<0.05$ and $p<0.001$ was considered as statistically significant in this study.

Results

The mean whole toenail selenium concentration was 0.58±0.13 $mg \cdot kg^{-1}$ (range: 0.29–1.08 $mg \cdot kg^{-1}$, n=331). The results found in the present study did not differ significantly between toenail selenium levels in individuals living in Pomeranian District (mean value: 0.57±0.10 $mg \cdot kg^{-1}$) and those determined in subjects from Lubuskie Districts (mean value: 0.60±0.16 $mg \cdot kg^{-1}$) ($p<0.05$). Table 2 shows the variables to be significantly related ($p<0.05$) to selenium levels.

In the population based study we found that gender, age, body mass index (BMI), smoking, and selenium supplementation are factors contributing to the apparent differences in the selenium levels in toenails.

It turned out that females from Pomeranian District and all females combined had statistically higher ($p<0.05$) selenium level than men. The differences in mean toenail selenium concentrations between genders within corresponding groups from Lubuskie District were, however, not significant.

An application of the Spearman test to toenail selenium levels and participants' age showed a statistically significant correlation (the Spearman correlation coefficient r=–0.28, $p<0.001$) (Fig. 1). The age above 50 years was associated with lower selenium concentration (Table 2). This pattern was also observed for both genders separately (Table 3).

Table 1. General characteristics of the study population

District	No. of subjects, n	Age ± s.d.	BMI[c] ± s.d.	No. of people taking supplements	No. of people dealing with smoking		
					current	ex-smokers	never
Lubuskie							
male	56	42 ± 18	26.0 ± 4.2	8	14	15	27
female	98	42 ± 18	24.3 ± 4.6	34	27	13	58
Pomeranian							
male	59	41 ± 17	27.2 ± 4.9	11	15	17	27
female	118	42 ± 16	24.4 ± 4.5	46	14	28	76

[c] Body mass index.

Table 2. Mean toenail selenium concentrations (mg·kg^{-1}) in Polish population according to different characteristics

Characteristic	All subjects			Pomeranian District			Lubuskie District			p^c
	n	x ± s.d.	p^a	n	x ± s.d.	p^a	n	x ± s.d.	p^b	
Gender			*0.0012*			*0.0026*			*0.50*	
male	115	0.57 ± 0.14		59	0.54 ± 0.10		56	0.59 ± 0.17		*0.086*
female	216	0.59 ± 0.12		118	0.582 ± 0.095		98	0.60 ± 0.15		*0.72*
Age			*0.0000*			*0.0022*			*0.0006*	
<25	64	0.62 ± 0.13		27	0.59 ± 0.10		37	0.64 ± 0.15		*0.11*
25–50	146	0.61 ± 0.14		82	0.59 ± 0.10		64	0.63 ± 0.17		*0.57*
>50	121	0.53 ± 0.10		68	0.533 ± 0.077		53	0.54 ± 0.13		*0.69*
BMI, kg·m^{-2}			*0.0005*			*0.060*			*0.0011*	
<18.5	13	0.69 ± 0.17		7	0.57 ± 0.10		6	0.82 ± 0.13		*0.0047*
18.5–25	181	0.59 ± 0.12		95	0.58 ± 0.10		86	0.60 ± 0.14		*0.61*
>25	137	0.56 ± 0.13		75	0.545 ± 0.090		62	0.58 ± 0.16		*0.79*
Smoking status			*<0.001*			*<0.001*			*<0.001*	
current	70	0.54 ± 0.12		29	0.53 ± 0.12		41	0.54 ± 0.13		*0.82*
ex-smoker	73	0.53 ± 0.10		45	0.526 ± 0.079		28	0.54 ± 0.13		*0.61*
never	188	0.62 ± 0.13		103	0.596 ± 0.090		85	0.65 ± 0.16		*0.050*
Supplements			*<0.001*			*<0.001*			*<0.001*	
user	99	0.66 ± 0.13		57	0.63 ± 0.10		42	0.71 ± 0.16		*0.026*
non-user	232	0.55 ± 0.11		120	0.537 ± 0.082		112	0.56 ± 0.14		*0.57*

[a] The p value for the differences in mean toenail selenium concentrations between variables for the whole study group.
[b] The p value for the differences in mean toenail selenium concentrations between variables for each district.
[c] The p value for the differences in mean toenail selenium concentrations between variables separately for both districts.

Table 3. The Spearman correlation between toenail selenium levels and age, and BMI for both groups of females and males

Characteristic	Females			Males		
	All (n=216)	PD[a] (n=118)	LD[b] (n=98)	All (n=115)	PD[a] (n=59)	LD[b] (n=56)
Age, years						
Significance level	*<0.001*	*0.022*	*0.0074*	*<0.001*	*<0.001*	*0.14*
Correlation coefficient	–0.25	–0.21	–0.27	–0.34	–0.49	–0.20
BMI, kg·m^{-2}						
Significance level	*0.0012*	*0.045*	*0.013*	*0.16*	*0.35*	*0.43*
Correlation coefficient	–0.22	–0.18	–0.25	–0.13	–0.12	–0.11

[a] Pomeranian District.
[b] Lubuskie District.

Higher age significantly influenced the toenail selenium concentration of all the females (r=–0.25) and males (r=–0.34). The analyses conducted separately for female and males residing in the Pomeranian and Lubuskie Districts revealed similar trends, merely the associations was not presented in men from Lubuskie District (Table 3).

Similarly, a significant negative correlation between selenium levels and body mass indexes (BMI) was observed (r=–0.22, p<0.001) (Fig. 2). In general, all participants combined with a BMI higher than 25 kg·m^{-2} had significantly lower toenail selenium concentration compared with those with lower value of BMI (p<0.05). The same was also true for subgroup consisting of participants from both districts studied, though the inhabitants of Pomeranian District did not show this difference as significant (Table 2). Subjects from Lubuskie District, with a BMI lower than 18.5 kg·m^{-2} exhibit significantly higher selenium level than remaining Lubuskie groups as well as the corresponding group from Pomeranian District. However, the number of subjects with BMI<18.5 kg·m^{-2} was much lower than the number of individuals with higher BMI. Moreover, since the underweight category (BMI<18.5 kg·m^{-2}) concerned only females, these findings may therefore need to be interpreted with the some amount of caution.

The reduction of selenium status in conjunction with increasing BMI was also significant for all females combined and females from Pomeranian and Lubuskie Districts. No differences were observed, however, for all males combined and the males living in each district (Table 3).

Regarding the impact of tobacco smoking on selenium levels, a significant effect (p<0.001) was found for the whole study group as well as for each district (Table 2). Generally, tobacco smoking was associated with lower selenium content (Fig. 3). The concentrations of Se in toenail samples of all nonsmokers, ex-smokers

and current smokers were found in the range of 0.40–1.08 mg·kg^{-1} (mean value: 0.62±0.13 mg·kg^{-1}), 0.29–0.88 mg·kg^{-1} (mean value: 0.53±0.10 mg·kg^{-1}), 0.35–0.92 mg·kg^{-1} (mean value: 0.54±0.12 mg·kg^{-1}), respectively. The non-smokers from Pomeranian District distinguished by lower mean toenail selenium level than those from Lubuskie District, although the differences was not significant (see Table 2).

The average selenium concentrations in each gender subgroup are shown in Table 4. The significant lower toenail selenium level (p<0.001) was more evident among the females than males. Furthermore, no significant differences were found between male non-smokers and both ex- and current male smokers from the Pomeranian District (p=0.12). Similar results were obtained for male subjects from the Lubuskie District. The significantly lower toenail selenium were only noted in male current smokers compared to never smokers (p<0.05), whereas no differences were found between non- and ex-male smokers (p=0.70).

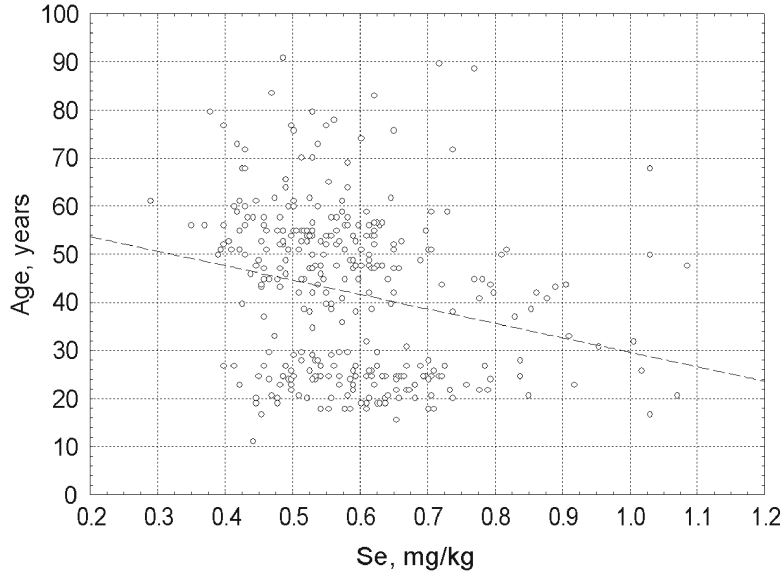

Fig. 1. Correlations of age and toenail selenium concentrations

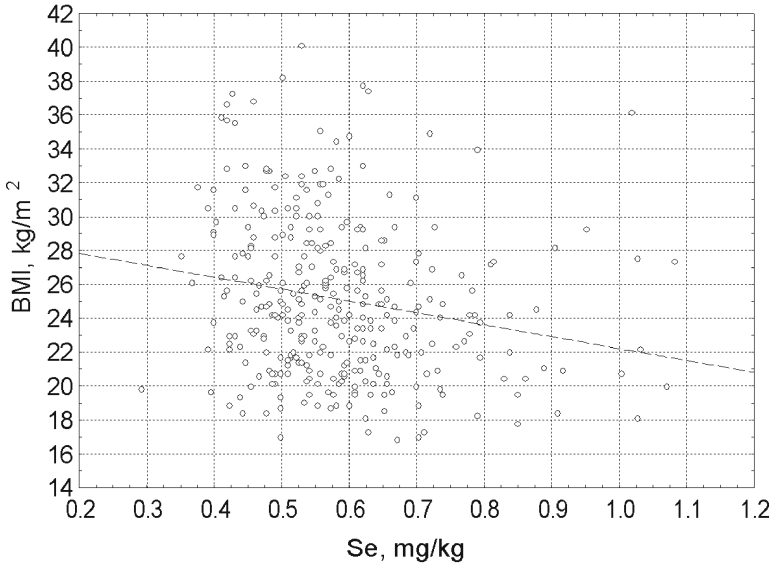

Fig. 2. Correlations of BMI and toenail selenium concentrations

The comparison of mean selenium concentrations in female non-smokers from Pomeranian District with the corresponding group from Lubuskie District did not show statistical differences (p>0.05). Similar results were also observed when such comparisons for the remaining subgroups between both districts were made.

A significant difference in selenium concentrations was observed between people taking the supplements and non-users (Table 2). The selenium supplement users from the Lubuskie District had significantly higher selenium concentration than the corresponding group from the Pomeranian District (p=0.026). Similarly, supplementation of selenium significantly influenced the toenail selenium level for both gender subgroups separately (p<0.001) (Table 4). The mean concentrations of Se in toenails of females and males taking the supplements (0.66±0.12 and 0.69±0.16 mg·kg^{-1}) were significantly higher than those determined in non-users (0.55±0.11 and 0.54±0.12 mg·kg^{-1}). The same was also observed when this comparison was made for females living in the Pomeranian and Lubuskie Districts. The increased selenium status due to the use of supplements was also significant for males living in Northern and Western Poland.

No significant differences (p>0.05) in selenium nail clipping levels were found for females from the Pomeranian District taking the supplements, and the corresponding group from the Lubuskie District. The same was observed during the similar comparison for the remaining subgroups in both districts.

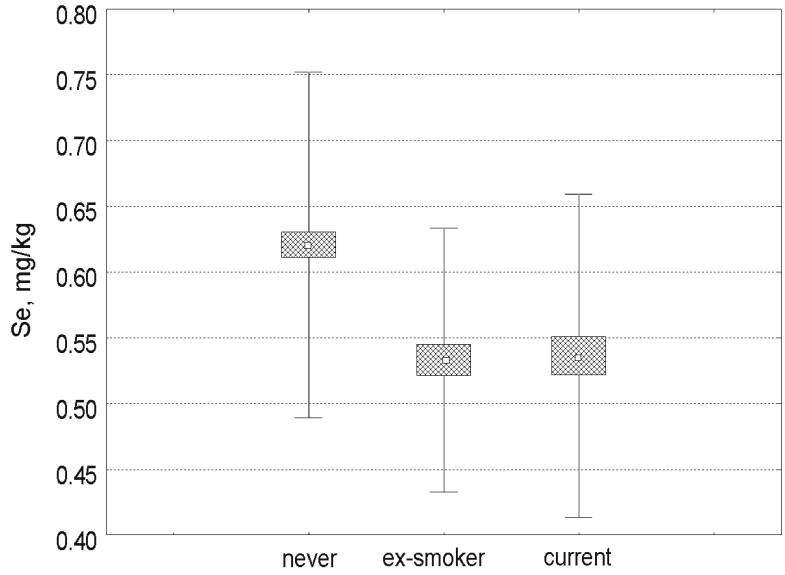

Fig. 3. Toenail selenium levels in all non-, ex-, and current smokers participated in the study

Table 4. Mean toenail selenium levels (mg·kg^{-1}) in both groups of females and males by smoking status and supplements use

Characteristic	Females			Males		
	All	PD[a]	LD[b]	All	PD[a]	LD[b]
	x ± s.d.	x ± s.d.	x ± s.d.	x ± s.d.	x ± s.d.	x ± s.d.
Smoking status						
current	0.54 ± 0.12	0.54 ± 0.13	0.54 ± 0.12	0.53 ± 0.12	0.53 ± 0.12	0.53 ± 0.13
ex-smoker	0.532 ± 0.080	0.540 ± 0.086	0.516 ± 0.068	0.53 ± 0.12	0.503 ± 0.061	0.56 ± 0.16
never	0.63 ± 0.12	0.605 ± 0.083	0.65 ± 0.16	0.61 ± 0.15	0.57 ± 0.10	0.64 ± 0.18
Supplements						
user	0.66 ± 0.12	0.633 ± 0.096	0.69 ± 0.15	0.69 ± 0.16	0.63 ± 0.12	0.77 ± 0.18
non-user	0.55 ± 0.11	0.549 ± 0.078	0.56 ± 0.13	0.54 ± 0.12	0.519 ± 0.084	0.56 ± 0.15

[a] Pomeranian District.
[b] Lubuskie District.

Discussion and conclusions

The overall average selenium concentration in toenail samples collected from the representatives of two Polish districts was found to be 0.58±0.13 mg·kg^{-1} which is comparable to those reported for residents of the Netherlands.[19] In comparison with other countries, the Se concentration level in our study was found to be lower than those of Canada, United States,[35,36] but higher than those of Helsinki's[37] populations. The results found in this study showed that the toenail selenium level of Polish population were below a diagnostic threshold defined by MORRIS et al.[38] Complying with this diagnostic we found that more than 90% of the studied population are at the increasing risk of chronic disease because of suboptimal selenium status. In the agreement with earlier findings presented by Polish researchers,[22] the obtained results may point out a need for supplementation with selenium.

In line with our hypotheses, a difference was observed in the concentrations of selenium in toenails from the inhabitants of Pomeranian and Lubuskie Districts, respectively. The mean value of toenail selenium content of the Pomerania inhabitants was found to be lower than that of the Lubuskie population. However, this difference was not statistically significant (p=0.24). It seems that the variation in toenail selenium based on place of residence might merely be due to the diversity of the environment and the related dietary intake via locally produced food. The remarkable correlation of supplementation and tobacco smoking with Se levels in nail clippings should be mentioned because of their consequences. The exclusion of the supplement users and smokers from the both groups have contributed to obtain comparable findings. For participants who neither took selenium supplements nor smoke there was no significant difference in toenail selenium concentration (p=0.72) between two geographic regions considered in the study.

A significant association of the demographic and behavioral characteristics on the toenail selenium levels was found. A positive significant relationship of female sex, younger age, underweight category, not smoking, and supplements use could be demonstrated with higher toenail selenium levels. The data presented are consistent with some previous studies but in contrast to other reports. HUNTER et al.[27] examined the predictors of toenail selenium within two subgroups of US women showed that the average toenail selenium was higher among consumers of selenium supplements than among non-users. Moreover, they revealed that the selenium nail clipping concentration was declined with age and was significantly reduced among cigarette smokers. The similar findings, but for each gender, was also observed by van den BRANDT et al.[19] An inverse association was noted between cigarette smoking and toenail selenium, indicating that current smokers had significantly lower selenium concentrations than those who never smoked or smoked in the past. Contrary to our findings, age was not the predictors for toenail selenium concentration. However, this study included the participants in the age range of 55 to 69 years for which we found the substantial decline of selenium status. MORRIS et al.[38] reported that gender, supplements use, and tobacco smoking were the major determinants of toenail selenium concentrations. Nevertheless, they showed no correlations between selenium level and age or BMI.

In conclusion, the presented data do not suggest that the Polish population is immediately endangered by an extremely low selenium status. However, the relatively low Se nail clipping levels may call for attention and consideration, especially if compared to the intervention Se values as defined by U.S. researchers; supplementation of selenium may be advisable. More studies are needed in Poland before such a decision may be taken, and this study is one of the first into this direction. Without any doubt, also systematic long term monitoring of the selenium status in Poland should be recommended.

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