

# A Low Selenium Level Is Associated with Lung and Laryngeal Cancers

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## Abstract

**Purpose:** It has been suggested that selenium deficiency is a risk factor for several cancer types. We conducted a case-control study in Szczecin, a region of northwestern Poland, on 95 cases of lung cancer, 113 cases of laryngeal cancer and corresponding healthy controls.

**Methods:** We measured the serum level of selenium and established genotypes for four variants in four selenoprotein genes (*GPX1*, *GPX4*, *TXNRD2* and *SEP15*). Selenium levels in the cases were measured after diagnosis but before treatment. We calculated the odds of being diagnosed with lung or laryngeal cancer, conditional on selenium level and genotype.

**Results:** Among lung cancer cases, the mean selenium level was 63.2 µg/l, compared to a mean level of 74.6 µg/l for their matched controls ( $p < 0.0001$ ). Among laryngeal cancer cases, the mean selenium level was 64.8 µg/l, compared to a mean level of 77.1 µg/l for their matched controls ( $p < 0.0001$ ). Compared to a serum selenium value below 60 µg/l, a selenium level above 80 µg/l was associated with an odds ratio of 0.10 (95% CI 0.03 to 0.34;  $p = 0.0002$ ) for lung cancer and 0.23 (95% CI 0.09 to 0.56;  $p = 0.001$ ) for laryngeal cancer. In analysis of four selenoprotein genes we found a modest evidence of association of genetic variant in *GPX1* with the risk of lung and laryngeal cancers.

**Conclusion:** A selenium level below 60 µg/l is associated with a high risk of both lung and laryngeal cancer.

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## Introduction

Selenium is a trace element which is an essential component of several major metabolic pathways, including the antioxidant defense system and the immune system [1,2]. Selenium intake varies, largely based on the selenium content of food. The mean selenium level of the population varies considerably between countries, with eastern European countries in general having levels that are much lower than those of Canada and the United States [1]. Several epidemiological studies have suggested inverse associations between serum selenium level and a number of different cancers, but the results have been inconsistent [3,4]. The data for a protective effect against lung cancer are among the most

compelling [3–10]. For many studies, the risk of cancer decreases with increasing serum selenium level, but the results of many other studies are negative and it is not clear if the dose-response curve is linear. In North America, where mean selenium levels are in the range of 120 µg/l - there may be less potential benefit to selenium supplementation than in countries where the mean level is low. The chemopreventive benefit of selenium may differ between individuals and the same dose may not be optimal for all. In supplementation trials, differences in the baseline level of selenium of patients or in the chemical form used as a selenium supplement (e.g. organic or inorganic) may also be important.

Some of the discrepancy in association studies might be explainable in part by the influence of genetic factors. Rayman

suggests that genetic variants in selenoproteins may be relevant [1]. Selenium is incorporated as selenocysteine at the active site of a number of proteins (selenoproteins) [11,12]. In humans there are 25 known selenoproteins, including four different glutathione peroxidases (*GPX1*, *GPX2*, *GPX3*, *GPX4*) and two thioredoxin reductases (*TXNRD1*, *TXNRD2*), but the function of many others is unknown [11,12]. Selenoproteins are involved in different biological processes, ranging from DNA synthesis to protection against oxidative stress. The latter is assumed to be the probable link with cancer risk; i.e. cancer associated with selenium deficiency may be attributed to increased oxidative stress and alterations in redox signaling due to selenoprotein impairment. To date, little information is available on the extent of genetic variability in selenoproteins within populations and the possible impact of particular genetic variants of selenoproteins on cancer risk. In the present study, we sought to identify common genetic variants among six selenoproteins in the Polish population and to evaluate whether or not these variants were associated with the risk of lung and laryngeal cancer. We asked also to what extent variation in the serum level of selenium contributed to the risks of lung and laryngeal cancer in Poland.

## Materials and Methods

### Study Participants

Lung cancer cases consisted of 95 consecutive patients with lung tumors resected at our center between August 2009 and January 2011. Laryngeal cancer patients consisted of 113 consecutive patients treated for laryngeal cancer diagnosed at our center between July 2009 and January 2011. Patients were not eligible to be cases if they were diagnosed with any malignancy in the past. Patients who were treated for cancer prior to blood sampling were also ineligible.

Controls were part of a population-based study of the 1.3 million inhabitants of West Pomerania designed to identify familial aggregations of cancer performed by our center. For selenium level estimation for each case, one control was selected. Controls were matched to cases with respect to year of birth (+/-3 years), sex, total number of lung or laryngeal cancers among first degree relatives, total number of cancers among first-degree relatives, and smoking (pack-years). It was possible to find a suitable control for 86 of the 95 lung cancer cases and for 87 of the 113 laryngeal cancer cases. For estimation of genetic polymorphisms in selenoprotein genes each of the 95 lung cancer and 113 laryngeal

cancer case was matched to 2 healthy controls. Matching included year of birth (+/-3 years), sex and smoking (pack-years). Cancer-free control individuals meeting matching criteria were identified by a review of the records of the population based study and invited for an interview and blood donation.

The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland and all participants gave informed written consent.

### Measurement of Selenium Level

Selenium concentration in blood plasma was determined in 86 lung cancer and 87 laryngeal cancer pairs using graphite furnace atomic absorption spectrometry (GFAAS). Measures were validated using reference material (lyophilized human reference serum samples of Serenoform™ from Nycomed Pharma AS, Oslo, Norway). For every four samples, a reference sample was measured to keep a constant control on the quality of the measurements. Cases and controls were tested alternately. The mean drift was used as a correction value for the samples, whereas a measured drift larger than 5% from the reference material caused the device to be re-calibrated and the previous samples to be re-measured. For 30 randomly selected individuals, the selenium measurement was performed in both plasma and serum. No differences in selenium level were observed depending on type of biological material studied.

### Sequencing of Selenoprotein Genes

As the initial step, we sought to identify common genetic variants in six selenoprotein genes: *GPX1*, *GPX2*, *GPX3*, *GPX4*, *TXNRD1* and *TXNRD2*. All exons and the flanking regions were sequenced in 50 unrelated Polish individuals. We identified 35 distinct sequence variants in the six genes. Three of these had a minor allele frequency in excess of 20% and were selected for further study (rs1050450 in *GPX1*, rs713041 in *GPX4* and rs1139793 in *TXNRD2*). One other variant (rs5845 in *SEPI5*) has been reported previously in association with cancer [13,14] and was also selected for further study. We then designed Realtime-PCR assays for each of the four candidate variants. The Realtime-PCR reaction was carried out with an LightCycler® 480 (Real-Time PCR System Roche diagnostics). Each variant defined one heterozygote and two homozygote genotypes. We then determined the genotypes for all 208 cases and 416 matched controls using these assays.

**Table 1.** Characteristics of Cases and Controls for Lung Cancer Study.

Characteristic	Cases (n = 86)	Controls (n = 86)	p-value
Mean year of birth (range)	1948.3 (1931–65)	1948.3 (1933–63)	0.95
Mean age at sample (range)	61.6 (46–78)	61.9 (47–77)	0.77
Sex	n (%)		
M (%)	41 (47.7%)	41 (47.7%)	
F (%)	45 (52.3%)	45 (52.3%)	
Smoking (past/current)			
No (%)	10 (11.6%)/60 (69.8%)	13* (15.1%)/60 (69.8%)	
Yes (%)	76* (88.4%)/26 (30.2%)	73 (84.9%)/26 (30.2%)	
Pack-years (range)	22.1 (0–80)	21.5 (0–70)	0.88
Selenium concentration in µg/l (range)	63.2 (29.0–105.6)	74.6 (32.4–115.7)	<0.0001

\*three never smoking controls were matched to lung cancer patients smoking <1pack-year.

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**Table 2.** Characteristics of Cases and Controls for Laryngeal Cancer Study.

Characteristic	Cases (n = 87)	Controls (n = 87)	p-value
Mean year of birth (range)	1949.9 (1929–69)	1950.4 (1931–68)	0.64
Mean age at sample (range)	59.9 (41–81)	59.9 (42–79)	0.97
Sex			
M (%)	73 (83.9%)	74 (85.1%)	
F (%)	14 (16.1%)	13 (14.9%)	
Smoking (past/current)			
No (%)	1 (1.2%)/43 (49.4%)	1 (1.2%)/39 (44.8%)	
Yes (%)	86 (98.8%)/44 (50.6%)	86 (98.8%)/48 (55.2%)	
Pack-years (range)	33.0 (0–60)	30.8 (0–75)	0.24
Selenium concentration in µg/l (range)	64.8 (33.6–114.6)	77.1 (44.6–129.3)	<0.0001

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## Statistics

The mean levels of selenium were compared for cases and controls using Student t-test. The associations between different categorical levels of selenium and disease status were evaluated by estimating odds ratios (OR) with 95% confidence intervals (CI) using conditional logistic regression for matched pairs. For these estimates, individuals were assigned to one of four categories, based on the distribution of selenium levels in tested groups, roughly corresponding to quartiles. The odds ratio was constructed with regard to the reference category, which was the lowest level. Each of the four tested variants was associated with three genotypes. The odds ratios for the two less frequent genotypes were generated using the most common genotype as the reference group. Samples with missing genotype in any of the four tested variants were excluded from analysis.

## Results

For the subjects in both the lung cancer and laryngeal cancer sub-studies, the cases and controls were well-matched for age, sex and smoking histories (Tables 1 and 2). Among controls (both subgroups combined) the mean serum selenium level was 75.2 µg/l for men and was 76.8 µg/l for women. The mean serum selenium level was 73.3 µg/l among current smokers, was 75.4 µg/l among past smokers and was 81.4 µg/l among never smokers. Among cases, the mean serum selenium level was 61.7 µg/l among current smokers, was 64.1 µg/l among past smokers and was 62.9 µg/l among never smokers.

Among lung cancer cases, the mean selenium level was 63.2 µg/l compared to a mean level of 74.6 µg/l for their matched controls ( $p < 0.0001$ ). Compared to a serum selenium value in the lowest category ( $\leq 60$  µg/l) a selenium level in the highest category ( $> 80$  µg/l) was associated with a risk reduction of

90% (OR 0.10; 95% CI 0.03 to 0.34;  $p = 0.0002$ ) for lung cancer (Table 3).

Among laryngeal cancer cases, the mean selenium level was 64.8 µg/l, compared to a mean level of 77.1 µg/l for their matched controls ( $p < 0.0001$ ). Compared to a serum selenium value in the lowest category ( $\leq 60$  µg/l) a selenium level in the highest category ( $> 80$  µg/l) was associated with a risk reduction of 77% (OR 0.23; 95% CI 0.09 to 0.56;  $p = 0.001$ ) for laryngeal cancer (Table 4).

In four selenoproteins studied here we found a modest associations of rs1050450 in *GPX1* with lung and laryngeal cancer risk. (Tables 5 and 6).

## Discussion

In our study, we sought to see if systemic differences in selenium levels occur in individuals with lung and laryngeal cancer and healthy controls in northwestern Poland. For both cancer sites, the cancer risk decreased with increasing levels of circulating selenium and the dose-response curve appeared linear. There was no evidence that there was a threshold above which the association with serum selenium was disrupted or reversed. The mean selenium level in the Polish control population was 75.8 µg/l. Only 21% (18/86) of the lung cancer cases and 28% (24/87) of the laryngeal cancer cases exhibited a level at or above this control mean. Compared to individuals in the category from 70 µg/l to 80 µg/l, those with a level below 60 µg/l were at roughly ten-fold increased risk for lung cancer and a three-fold increased risk of laryngeal cancer.

The mean level of selenium in the controls was less than optimal according to the proposed level of 120 µg/l to be optimal for proper selenoprotein expression and activity [1]. In our study, only one control (and none of cases) had a level that exceeded 120 µg/l.

**Table 3.** Serum selenium levels and the risk of lung cancer.

Selenium level	Cases (%) n = 86	Controls (%) n = 86	OR (95% CI)	p-value
$\leq 60$	37 (40.0)	12 (14.0)	1	
61–70	23 (26.7)	18 (20.9)	0.40 (0.15–1.09)	0.07
71–80	12 (14.0)	29 (33.7)	0.11 (0.03–0.33)	0.0001
$> 80$	14 (16.3)	27 (31.4)	0.10 (0.03–0.34)	0.0002

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**Table 4.** Serum selenium levels and the risk of laryngeal cancer.

Selenium level	Cases (%) n = 87	Controls (%) n = 87	OR (95% CI)	p-value
≤60	33 (37.9)	13 (14.9)	1	
61–70	23 (26.4)	16 (18.4)	0.59 (0.21–1.65)	0.32
71–80	15 (17.2)	23 (26.4)	0.35 (0.14–0.87)	0.02
>80	16 (18.4)	35 (40.3)	0.23 (0.09–0.56)	0.001

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Several case-control and cohort studies have been conducted up to date to assess the influence of selenium on lung cancer risk. Some of these studies suggested that selenium may have a protective effect against lung cancer [15–18]. However, other studies have failed to confirm this observation, either showing no association [19–23] or an adverse effect [24,25] of higher selenium level on incidence of lung cancer. These studies differed in study design (population or nested-case-control, with or without selenium supplementation), low or high baseline population selenium level, methods of selenium measurements (dietary intake, toenail or serum level) and the length of the follow-up period. A meta-analysis of 16 studies on selenium and lung cancer showed the pooled RR 0.74 (95% CI 0.57–0.97) for subjects with higher selenium exposures. The protective effect was greater for studies from areas with lower average baseline selenium level (RR 0.72) than from areas with higher average selenium level (RR 0.86) [26]. The possibility that the protective effect of selenium may be limited to individuals with a low baseline level is supported by the NPC trial [8,9]. In the NPC study, American subjects were randomized to receive either selenium 200 µg/day versus placebo [8]. After 7.9 years of follow-up, the odds ratio for incident lung cancer was 0.70 (95% 0.40 to 1.21;  $p = 0.18$ ). Notably, the benefit was limited to individuals with low baseline plasma selenium (<106 µg/l). In this subgroup the association with selenium supplementation was 0.42 (95% CI 0.18 to 0.96). Of interest, at this cutoff point, 96% (166/173) of Polish controls would fall into

this category. In the recently published SELECT trial [27], there was no benefit for men randomized to selenium 200 µg/l versus placebo in terms of incident lung cancers (HR 1.12; 95% CI 0.73 to 1.72). However, in that study, the mean baseline selenium level was 137 µg/l and data were not subdivided by serum selenium.

There are several limitations to our study. Perhaps the most important limitation of our case-control study is that the selenium measurements for cases were made after the diagnosis of lung or laryngeal cancer, but before therapy. It has been reported that acute and chronic injury and infections alter concentration of variety of micronutrients [28,29]. As in this study the selenium concentration in cancer patients was measured after they were diagnosed, it is possible that the detected low selenium level could be a consequence of their disease. If this were the cases, then a low selenium level would be a marker of risk rather than a risk factor.

The sample size in our study is relatively small but the associations were strong and highly significant. The cases and controls were matched on smoking status and it is therefore not likely that the association could be confounded by smoking, but it is possible that another confounding variable could introduce bias.

In four selenoproteins studied here we found some evidence of modest association of genetic variant in *GPXI* with lung and laryngeal cancer risk. The influence of rs1050450 in *GPXI* on lung cancer risk have been already analyzed in several studies, but results were inconsistent [30–34]. In the current study we found an indication of association of T-allele with reduction of lung and

**Table 5.** Genotypes for selected selenoproteins and the risk of lung cancer.

	Cases (%) n = 95	Controls (%) n = 176	OR (95% CI)	p-value
<i>GPXI</i> (rs1050450)				
CC	53 (55.8)	79 (44.9)	1	
CT	33 (34.7)	83 (47.2)	0.54 (0.31–0.95)	0.03
TT	9 (9.5)	14 (7.9)	0.85 (0.33–2.19)	0.73
<i>GPX4</i> (rs713041)				
CC	39 (41.1)	54 (30.7)	1	
CT	47 (49.5)	97 (55.2)	0.71 (0.41–1.22)	0.21
TT	9 (9.5)	25 (14.2)	0.47 (0.19–1.17)	0.11
<i>SEP15</i> (rs5845)				
GG	54 (56.8)	100 (56.8)	1	
AG	35 (36.8)	68 (38.6)	1.03 (0.62–1.72)	0.91
AA	6 (6.3)	8 (4.5)	1.52 (0.51–4.5)	0.45
<i>TXNRD2</i> (rs1139793)				
GG	55 (57.9)	109 (61.9)	1	
AG	33 (34.7)	56 (31.8)	1.16 (0.69–1.95)	0.58
AA	7 (7.4)	11 (6.3)	1.27 (0.49–3.32)	0.63

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**Table 6.** Genotypes for selected selenoproteins and the risk of laryngeal cancer.

	Cases (%) n = 111	Controls (%) n = 213	OR (95% CI)	p-value
<i>GPX1</i> (rs1050450)				
CC	65 (58.6)	97 (45.5)	1	
CT	37 (33.4)	95 (44.6)	0.51 (0.29–0.9)	0.02
TT	9 (8.1)	21 (9.8)	0.83 (0.28–2.41)	0.73
<i>GPX4</i> (rs713041)				
CC	42 (37.8)	68 (31.9)	1	
CT	46 (41.5)	108 (50.7)	0.9 (0.5–1.61)	0.72
TT	23 (20.7)	37 (17.4)	1.15 (0.53–2.48)	0.73
<i>SEP15</i> (rs5845)				
GG	61 (54.9)	128 (60.0)	1	
AG	47 (42.4)	73 (34.3)	1.51 (0.85–2.66)	0.16
AA	3 (2.7)	12 (5.6)	0.4 (0.08–2.06)	0.27
<i>TXNRD2</i> (rs1139793)				
GG	57 (51.4)	117 (54.9)	1	
AG	44 (39.6)	82 (38.5)	1.26 (0.73–2.18)	0.41
AA	10 (9.0)	14 (6.6)	1.72 (0.55–5.34)	0.35

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laryngeal cancer risk, however these results were of borderline significance.

Our study indicates that a low selenium level is significantly associated with up to a tenfold higher risk of lung and laryngeal cancer for individuals in the lowest category of selenium. Ideally this association would be confirmed in a large prospective study. If confirmed, it is possible that low serum selenium level will appear to be a strong indicator for the risk of lung and laryngeal cancer, then this will be an important potential target of therapeutic intervention.

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## Author Contributions

Collected blood samples and clinical informations from lung cancer patients: TG PW JW. Collected blood samples and clinical informations from laryngeal cancer patients: EJ MS Jakub Lubiński. Collected blood samples and clinical informations from healthy controls: KJ SG KD MM GS CC TD ML KK. Revised critically and approved final of the version to be published: KJ SG KD MM GS EJ TG MS PW JW Jakub Lubiński CC TD ML AWM KK PS Jan Lubiński. Conceived and designed the experiments: KJ SG Jan Lubiński AJ. Performed the experiments: KJ SG KD MM GS EJ TG MS PW JW Jakub Lubiński CC TD ML AWM AJ. Analyzed the data: KJ SG KD SAN PS Jan Lubiński AJ. Contributed reagents/materials/analysis tools: MM GS EJ TG MS PW JW Jakub Lubiński CC TD ML AWM KK Jan Lubiński AJ. Wrote the paper: SAN AJ.

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