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Serum selenium and glutathione peroxidase concentrations in healthy Iranian subjects

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Abstract.
BACKGROUND AND AIM: We have investigated the association between serum selenium and GPx concentrations, demographic characteristics, anthropometric features, fasting lipid profile and blood glucose levels, in healthy subjects.
METHODS: Serum selenium was measured by atomic absorption spectrometry in 197 healthy subjects. Serum glutathione peroxidase, fasting lipid profile, and blood glucose levels were also determined for each subject. Anthropometric features including blood pressure and body mass index were determined using standard procedures.
RESULTS: The mean serum selenium in the whole subjects group was 116 ± 27.73 μg/l (range 44–209 μg/l). Serum Selenium and GPX concentration did not vary significantly with gender and smoking habit. Significant differences were observed in serum selenium and GPx concentration with age ($P < 0.05$ and $P < 0.02$ respectively). Obese subjects had significantly lower serum concentrations of GPx ($r = 0.281$, $P = 0.01$). Serum GPx concentrations were inversely related to weight ($P = 0.01$), systolic blood pressure ($P < 0.01$), fasting blood sugar ($P < 0.01$), serum low-density lipoprotein-cholesterol (LDL-C) ($P = 0.01$), serum triglycerides ($P < 0.01$), and positively associated with fasting total cholesterol levels. The corresponding predictors for serum selenium level were diastolic blood pressure ($P < 0.05$) and serum triglycerides level ($P = 0.05$), which were inversely related.
CONCLUSION: Serum concentration of selenium in an Iranian healthy population is higher than most countries however, similar to the US and UK. Moreover, serum selenium and GPx concentrations appear to be influenced by physiological factors including age. Their serum concentrations were also associated with coronary risk factors, including serum total cholesterol and serum triglycerides levels.

Keywords: Selenium, GPx, healthy adults

1. Introduction

Selenium is an important trace element; selenium is part of the active site of the enzyme glutathione peroxidase antioxidant (GSH-Px). Selenium is a modulator of the response to oxidative stress and selenium supplements have been reported to result in a faster restoration of the endogenous antioxidative defense system against the production of

[1]Two authors regard as same contribution.

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reactive oxygen species [1]. Free radicals are thought to play an important role in the pathogenesis of coronary heart disease (CHD) and carcinogenesis [2, 3]. Epidemiological studies show an inverse relationship between selenium intake and cancer incidence, and mortality [4]. Furthermore, selenium has a narrow therapeutic range [5] and high selenium concentrations have been associated with increased lipid levels [6] and higher prevalence of hypertension [7] and diabetes [8, 9]. However, the interpretation of these studies may be confounded by variables that contribute to disease risk, that also affect trace element status [10]. Therefore, determining the relationship between a number of factors that have been reported or may be expected to affect trace element status and serum selenium concentration may contribute to better assessing the health and nutritional status of certain population. In the present study, we aimed to evaluate the influence of demographic characteristics and lifestyle factors on plasma levels of selenium in the adult population in Iran.

2. Material and methods

2.1. Subjects

Approximately 197 healthy subjects (80 men, 117 women; mean 38.5 age: years, range: 18–80 years) were enrolled in our study. The socio-economic status of all subjects corresponded to the middle-class Iranian population. They had comparable level of activity. Subjects who were on vitamin supplements, lipid-lowering medications, oral contraceptives, or hormone replacement therapy were excluded from the study. Pregnant women were also excluded. All subjects gave informed written consent to participate in the study, which was approved by Mashhad University of Medical Science Ethics Committee. In addition, a standardized questionnaire was also filled-out by individuals to provide information on age, gender, socioeconomic status and smoking habits, etc.

2.2. Anthropometric measurement

For anthropometric measurements including weight and height standard protocol were used. The subjects were shoeless and dressed in very light clothing after overnight fast. Body weight was measured with a portable digital standard scale to accuracy of ± 0.1 Kg, and height was measured with a portable standiometer to an accuracy of ± 0.1 cm. Body mass index (BMI) was calculated as weight divided by height square2 (m).

2.3. Blood collection

Blood samples were collected in the morning from each subject after overnight fast by venepuncture of the antecubital vein. After being allowed to clot, it was centrifuged at 2500 rpm for 15 min at room temperature to obtain serum, taking care to avoid possible sources of trace element contamination. Serum was stored at -20°C before analysis.

A full-fast lipid profile was determined for each subject. LDL cholesterol was calculated using the Friedewald equation, except for subjects with triglyceride concentrations of >4.0 mmol/L. Serum lipid and fasting blood sugar concentrations were measured by enzymatic methods.

Selenium was determined by electro-thermal atomic absorption spectrometry with Zeeman background correction using a palladium chloride chemical modifier [11].

2.4. GPx assay

Serum GPx was measured using a modification of the method of Paglia and Valentine [12]. Briefly, 10 mL of serum, standard (0.1–0.3 U/mL purified GPx) or water (blank) was added in quadruplicate to a 96-well plate. In all, 290 μL of 0.1 mol/L phosphate buffer (containing 5 mmol/L EDTA, 200 μmol/L sodiumazide, 1 U/mL glutathione reductase, 0.86 mmol/L NADPH, 2 mmol/L reduced glutathione and 7.8 mmol/L t-butyl hydroperoxide) was added

to each well. The reagents were mixed and the absorbance at 340 nm measured continuously for 5 min in an iEMS MF plate reader. The between assay CV was typically 5.5%.

2.5. Statistical analysis

The data were subjected to statistical evaluation using MiniTab (release 13, Minitab Inc, 2000, USA), with descriptive statistics (mean, medium, standard deviation [SD], and interquartile range) being determined for all variables. The Kolmogorove-Smirnov's test was carried out to examine if the variables had a normal distribution ($P < 0.05$). The mean values of different groups were compared by one-way ANOVA and Student's *t*-test. Categorical data were compared using Fisher's exact or χ^2 tests. Stepwise multiple regression analysis was used to predict whether serum trace element was related to factors such as age, gender, smoking, BMI, fasting blood glucose, lipid profile, and systolic and diastolic blood pressure. *P* value <0.05 was considered significant.

3. Results

A total of 197 healthy persons were enrolled in our study, of this group, 80 (40.6%) were men and 117 (59.4%) were women. The mean age of subjects was 38.5 ± 16.5 years (Table 1). The mean serum selenium and GPx concentrations in the whole subjects group was 116 ± 27.73 μg/l (range 44–209 μg/l) and 0.31 ± 0.072 μg/l (range 0.18–0.52 μg/l), respectively.

No significant differences between the sexes were observed in the mean values of serum selenium and GPx concentrations ($P = 0.319$ and $P = 0.194$, respectively), although serum concentrations of selenium and GPx tended to present higher values among the females (117.80 ± 27.20 vs 113.80 ± 28.30 μg/l and 0.32 ± 0.08.vs 0.30 ± 0.05 μg/l, respectively) (Table 1).

Table 1
Characteristics of the study population by gender and smoking status

	Gender		Smoking	
	Female	Male	Female	Male
Total population n(%)	117(59.4%)	80(40.6%)	47(23.9%)	150(76.1%)
Age (years)	37.63 ± 42.53	18.02 ± 17.02	39.29 ± 17.75	42.63 ± 17.19¥
Height (cm)	161.85 ± 7.45	166.28 ± 8.61*	163.13 ± 8.50	166.15 ± 7.77
Weight (kg)	66.13 ± 14.08	70.77 ± 13.76¥	67.86 ± 14.48	70.83 ± 12.38
BMI (kg/m2)	25.00 ± 4.91	26.08 ± 4.48	25.58 ± 4.88	25.67 ± 4.24
Waist (cm)	90.21 ± 17.55	91.35 ± 12.81	90.49 ± 16,27	93.46 ± 12.69
Hip (cm)	99.90 ± 9.54	99.20 ± 9.74	99.76 ± 9.98	100.14 ± 8.66
Waist/hip	0.89 ± 0.12	0.91 ± 0.09	090 ± 0.11	0.92 ± 0.08
SBP (mmHg)	110 ± 15.44	112 ± 15.40	115.23 ± 15.92	113.45 ± 14.83
DBP (mmHg)	70.49 ± 11.88	72 ± 9.44	73.52 ± 10.92	72.35 ± 8.17
FBS (mg/dL)	85.10 ± 14.22	86.62 ± 21.37	86.46 ± 18.83	84.63 ± 13.17
LDL (mg/dL)	110.87 ± 32.85	110.62 ± 34.03	110.99 ± 33.13	111.23 ± 34.76
HDL (mg/dL)	41.58 ± 7.25	39.97 ± 8.88	41.50 ± 7.64	39.22 ± 8.80
TG (mg/dL)	129.53 ± 98.86	128.75 ± 83.46	126.16 ± 86.34	186.91 ± 52.91
TC (mg/dL)	181.73 ± 48.49	178.64 ± 50.52	181.05 ± 48.79	186.91 ± 52.91
Selenium level (μg/L)	117.80 ± 27.20	113.80 ± 28.30	117.10 ± 29.90	115.9 ± 27.10
GPx (U/mL)	0.32 ± 0.08	0.30 ± 0.05	0.3162 ± 0.08	0.3161 ± 0.07

Values are expressed as mean ± SD. Between smoking status and gender groups comparisons were assessed by *t*-test. *$P < 0.001$. **$P < 0.01$. ¥$P < 0.05$. BMI, body mass index; FBS, fasting blood sugar; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GPx, glutathione peroxidase; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.

Table 2
Characteristics of the study population by age status

	Age (years)					
	18–25	26–35	36–45	46–55	56–65	66–80
Total population n(%)	70(35.5%)	15(7.6%)	45(22.8%)	29(14.7)	32(16.2%)	6(3%)
Male/Female	13/17	4/9	24/21	16/15	16/17	1/4
Smoker/ Nonsmoker	8/21	5/8	11/34	7/24	10/23	1/4
Height (cm)	165.17 ± 9.47	161.42 ± 5.81	165.31 ± 8.30	167.08 ± 7.50	167.08 ± 7.50	167.08 ± 7.50
Weight (kg)	61.48 ± 13.27	68.60 ± 10.43	73.90 ± 12.46	74.95 ± 10.36	73.26 ± 11.03	76.20 ± 16.67*
BMI (kg/m^2)	21.86 ± 3.56	26.23 ± 3.48	27.26 ± 4.78	26.95 ± 3.76	27.61 ± 3.91	28.40 ± 4.19*
Waist (cm)	79.02 ± 10.55	90.65 ± 9.21	95.16 ± 10.06	98.59 ± 9.60	99.93 ± 11.78	116.20 ± 35.00*
Hip (cm)	95.35 ± 7.32	101.46 ± 7.04	103.45 ± 7.10	103.03 ± 8.34	100.81 ± 10.19	104.600 ± 7.43*
Waist/Hip	0.82 ± 0.69	0.89 ± 0.074	0.91 ± 0.05	0.95 ± 0.08	0.99 ± 0.08	1.09 ± 0.25*
SBP (mmHg)	103.85 ± 13.18	108.46 ± 10.68	115.73 ± 11.93	117.35 ± 12.37	125.81 ± 11.71	128.00 ± 11.51*
DBP (mmHg)	65.53 ± 10.02	71.30 ± 8.82	73.37 ± 8.82	74.03 ± 10.83	78.00 ± 9.60	80.00 ± 9.35*
FBS (mg/dL)	76.96 ± 11.29	78.53 ± 8.53	82.66 ± 17.05	92.74 ± 11.99	97.75 ± 25.22	85.50 ± 18.73*
LDL (mg/dL)	91.26 ± 20.50	90.79 ± 24.51	121.33 ± 33.54	133.38 ± 26.17	127.13 ± 29.37	134.33 ± 34.42*
HDL (mg/dL)	37.96 ± 6.13	35.15 ± 8.47	41.40 ± 8.57	44.66 ± 5.47	42.39 ± 8.13	39.66 ± 2.08*
TG (mg/dL)	74.03 ± 35.48	105.69 ± 73.40	136.38 ± 89.04	165.77 ± 96.53	183.18 ± 128.69	159.25 ± 63.05*
TC (mg/dL)	141.03 ± 25.05	147.07 ± 24.99	189.97 ± 36.93	217.92 ± 48.04	214.00 ± 48.76	219.75 ± 72.53*
Selenium level (μg/L)	124.80 ± 18.30	123.4 ± 20.3	112.1 ± 32.4	105.6 ± 34.5	109.5 ± 30.5	115.6 ± 16.7**
GPx (U/mL)		0.33 ± 0.05	0.30 ± 0.06	0.26 ± 0.04	0.27 ± 0.05	0.27 ± 0.02*

Values are expressed as mean ± SD. Comparison between age categories using one-way ANOVA. *$P<0.001$. **$P<0.01$. ***$P<0.05$. GPx, glutathione peroxidase; BMI, body mass index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FBS, fasting blood sugar; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Of the participants, 47(23.9%) were smoker (Table 1). The mean serum selenium and GPx level of smokers (117.10 ± 29.90 and 0.30 ± 0.05 μ g/l, respectively) was lower than that of non-smokers (115.9 ± 27.10 and 0.3161 ± 0.07 μg/l, respectively), although this was not statistically significant ($P=0.811$ and $P=0.998$, respectively) (Table 1).

Participants were divided into six subgroups based on their age. The highest concentrations of selenium and GPx occurred at the age group of 18–25 years old (124.8 ± 18.30 μg/l and 0.36 ± 0.07 μg/l, respectively). Serum selenium and GPx concentrations were lowest in 46–55 years age group (105.6 ± 34.5 and 0.26 ± 0.04, respectively). The statistically significant difference for selenium and GPx concentrations, among different age groups is found by conducting one-way ANOVA analysis ($P=0.01$, $P<0.0001$, respectively) (Table 2).

In our study, 31(16.35%) were obese (BMI >30), 68(35.85%) were overweight (25< Bp;MI ≤30), 83(43.7%) were normal (18< BMI ≤25) and 8(4.2%) were underweight (BMI ≤18). Serum selenium concentrations fell with increasing BMI category up to obese people (BMI >30), it was highest in BMI ≤18 and BMI > 30 (128.7 ± 18.5 and 123.90 ± 35.5, respectively) compared other BMI categories. The difference was statistically significant ($P=0.004$). The highest and lowest GPx concentrations were in BMI <18(0.36 ± 0.08) and 25< BMI ≤30 (0.29 ± 0.04), respectively. There were no significant correlations between these BMI categories and GPx level ($P=0.064$).

3.1. Univariate analysis of factors associated with serum selenium concentration

There were weak but negatively significant association between serum selenium concentrations and age ($r=-0.205$, $P=0.006$), triglycerides ($r=0.156$, $P=0.038$) and cholesterol ($r=-0.229$, $P=0.003$). Serum GPx levels were negatively associated with weight ($r=-0.281$, $P=0.01$), BMI ($r=-0.227$, $P=0.04$), SBP ($r=-0.447$, $P=0.00$), FBS ($r=-0.229$, $P=0.00$), LDL ($r=-0.283$, $P=0.01$), and positively associated with cholesterol ($r=0.321$, $P=0.00$) (Table 4).

Table 3
Characteristics of the study population by BMI status

	BMI (kg/m^2)			
	<18 (underweight)	>18<25(normal)	>25<30(overweight)	30<(obese)
Total population n (%)	8(4.2%)	83(43.7%)	68(35.85%)	31(16.35%)
Age (years)	28.42 ± 26.60	34.25 ± 16.92	47.34 ± 13.21	49.70 ± 14.31
Male/Female	4/3	32/39	35/31	11/20
Smoker/ Nonsmoker	1/6	17/52	23/43	11/20
Height (cm)	156.22 ± 11.72	165.03 ± 7.99	165.49 ± 7.82	160.50 ± 7.83*
Weight (kg)	41.84 ± 7.79	60.64 ± 8.89	73.77 ± 7.79	85.79 ± 10.88*
Waist (cm)	67.82 ± 10.76	82.93 ± 9.72	96.11 ± 8.16	110.00 ± 16.26*
Hip (cm)	79.92 ± 13.28	95.59 ± 5.35	101.42 ± 7.03	111.82 ± 6.58*
Waist/Hip	0.85 ± 0.15	0.86 ± 0.09	0.94 ± 0.08	0.97 ± 0.13*
SBP (mmHg)	92.14 ± 22.30	107.75 ± 14.58	117.77 ± 13.42	123.09 ± 13.27*
DBP (mmHg)	59.28 ± 11.70	67.45 ± 8.71	75.04 ± 10.30	77.25 ± 10.63*
FBS (mg/dL)	73.57 ± 19.24	80.90 ± 12.36	89.81 ± 19.01	90.22 ± 22.22*
LDL (mg/dL)	85.20 ± 26.23	104.76 ± 31.40	115.22 ± 30.73	112.75 ± 33.11*
HDL (mg/dL)	42.83 ± 11.40	40.11 ± 6.89	40.55 ± 8.90	42.67 ± 7.57
TG (mg/dL)	92.33 ± 34.90	99.22 ± 66.92	156.07 ± 113.46	165.16 ± 90.97*
TC (mg/dL)	155.42 ± 33.98	167.19 ± 46.07	192.66 ± 48.50	210.40 ± 49.85*
Selenium level (μg/L)	128.7 ± 18.5	118.3 ± 21.2	106.4 ± 29.2	123.90 ± 35.5**
GPx (U/mL)	0.36 ± 0.08	0.32 ± 0.07	0.29 ± 0.04	0.30 ± 0.08

Values are expressed as mean ± SD. Comparison between BMI categories using one-way ANOVA. *$P < 0.001$. **$P < 0.01$. ***$P < 0.05$. GPx, glutathione peroxidase; BMI, body mass index; FBS, fasting blood sugar; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.

Table 4
Correlation (r) between indices of serum selenium status and coronary risk factors

	Selenium	GPx
	r	r
Age	−0.205**	−0.372*
Height	−0.101	−0.169
Weight	−0.122	−0.281**
BMI	−0.088	−0.227***
Waist	0.086	0.237
Hip	0.030	0.039
Waist/Hip	−0.108	0.337*
Systolic BP	0.106	−0.447*
Diastolic BP	−0.108	−0.229
FBS	0.012	−0.229*
TC	−0.229**	0.321*
LDL	−0.075	−0.283**
HDL	0.060	−0.121
TG	−0.156¥	−0.301*

Correlation were assessed using Pearson correlation coefficients; *$P < 0.001$. **$P < 0.01$. ***$P < 0.05$. GPx, glutathione peroxidase; BMI, body mass index; BP, blood pressure; FBS, fasting blood sugar; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 5
Comparison of the mean serum levels of selenium, among adults from different countries (18, 20)

Country	Overall mean (μg/l)
Finland (Helsinki)	41.7
New Zealand (Dunedin)	47.2
Brazil (Rio de Janeiro)	73.2
W. Germany (Mainz)	81.1
Sweden (lund)	85.0
Italy (Rome)	89.8
Japan (Hiroshima)	97.6
Saudi Arabia	102.5
US (Mort.Gr.)	110.2
England (Southampton)	115.7
Iran(Mashhad)	116.0
Canada (Toronto)	158.3

1 μg = 0.0127 μmol.

Our findings demonstrate groups at risk of deficiency, and suggest factors that may influence plasma levels of selenium.

4. Discussion

Selenium is involved in many biochemical processes supporting life. The most important of these processes are cellular respiration, DNA and RNA reproduction, maintenance of cell membrane integrity, and sequestration of free radicals. Selenium is involved in destruction of free radicals through cascading enzyme systems. Hydrogen peroxide is then reduced to water by the selenium-glutathione peroxidase couple. Efficient removal of these superoxide free radicals maintains the integrity of membranes, reduces the risk of cancer, and slows the aging process. Trace element-deficient subjects usually present with common symptoms such as malaise, anemia, infection, skin lesions, and low-grade neuropathy [13]. A lot of studies showed relationship between selenium deficiency and disease, and support this concept [14–17].This is the first study that has identified various factors which may contribute to a selenium status in a nationally representative sample of Iranian women and men.

The mean serum selenium level for adults observed in this survey was 116 ± 27.73 μg/mLwhich was similar to the one described in an exploration in the England and US [18]. In the Nutritional Prevention of Cancer (NPC) Trial [19], a serum selenium level of 80 ng/mL is reflected the minimum level of serum selenium essential in the bloodstream for full construction of seleno-proteins. The table reviews the selenium serum levels in the Iranian population in comparison with different national population data.

Previous studies have reported that serum selenium is not significantly related to gender, although others have reported that healthy women have higher serum selenium concentrations than their male counterparts [21–24]. We did not find significant difference in serum selenium concentrations related to gender. Whether there is a gender difference in serum GPx activity remains controversial [25, 26]. Our study had also failed to find any significant association between gender and serum GPx activity.

Some studies have previously reported that within a healthy population serum selenium concentration are reduced in smokers [27].While other investigators did not show any relationship between serum selenium concentrations and smoking [28]. In our study, we found no significant differences in serum concentrations of selenium or GPx between smoker subjects and non-smokers. This may be dependent on the type and form of tobacco consumption, which is likely to differ between the two populations.

By other authors, significant variation with regard to age has also been reported [29–31]. However, we did find a negative correlation between serum Se concentration and age until 50 years old. The serum selenium content in Iranian would increase with age when they were older than 50. This decline in younger population of Iranian peoples

may be as a result of food habit change in this group of subjects. Adolescent and more elderly population was more likely to maintain the traditional diet, with a significantly higher consumption fruit, vegetables and carbohydrates, and a lower intake of sweets.

Other investigators have reported that serum selenium concentrations are significantly lower in obese subjects [32], while other studies have reported no significant difference in serum Se concentration between obese and non-obese subjects [10]. In our study, serum Se concentration found to be significantly higher in obese Iranian subjects. Like some previous studies, we found lower serum GPx concentrations in obese peoples compared to non-obese subjects [33].

Some previous studies have found no significant association between serum Se and several coronary risk factors [34]. Other investigators reported a positive and significant relationship serum Se and serum cholesterol, and serum GPx and HDL cholesterol consentrations [10]. Our study did show weak association between serum Se concentration and serum cholesterol and triglycerides Level. There was negative association between serum GPx and weight, BMI, SBP, FBS and LDL cholesterol concentrations, and positive association between serum GPx and serum cholesterol level.

5. Conclusion

Supplementation among people with low selenium status has been reported to decreased the incidence rate of various type of illness from prostate cancer to viral infection [35]. On the other hand, for individuals living in region with high selenium intake, selenium supplementation could potentially increase risk of hypertention, diabetes, or hypercholesterolemia [7–9]. These findings call for a thorough evaluation of the risks and benefits associated selenium supplementation. The result of our study, could be used to identify people who have risk factor for low selenium status and would benefit from blood test, if their test determines low serum selenium concentration, they should prescribed a daily selenium supplement and recommended to modify their lifestyle. So that the diseases associated with selenium deficiency can be prevented and their incidences can be then reduced. By this approach, we would eliminate the possibility of toxicity and side effects of selenium supplementation.

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Declaration of interest

The authors have no conflict of interests to declare.

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