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Urinary Selenium Levels in Japanese Males and Females

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In the investigation of selenium metabolism, the analysis of urinary selenium is essential, since selenium is excreted primarily via urine (Levander et al. 1981; Swanson et al. 1983). Its level in the urine has been found to correlate considerably with that in blood plasma (Wasowicz and Zachara 1987). Many investigators have reported the urinary selenium levels of healthy people in various countries (Mondragon et al. 1971; Fell et al. 1981; Alexander et al. 1983; Palmer et al. 1983; Wasowicz et al. 1988). Although some investigators reported no difference between the values for male and female, their investigations were limited in the age of subjects (Hongo et al. 1985) and/or the number of samples (Wasowicz and Zachara 1987).

To elucidate the relationship between urinary selenium level and age or sex, we collected 789 single-void urine samples from inhabitants of Sagamihara area, nearby Tokyo. More than 30 samples constituted each age class of 10 year-interval for both sexes. To analyze such a large number of samples, we have developed a microfluorometric method (Hasunuma et al. 1982).

Mondragon et al.(1971) and Yang et al.(1983) have found remarkable differences in urinary selenium levels due to the locality of the samples in their countries. Therefore, we examined urine samples from 9 districts throughout Japan and found a little significant differences among districts examined.

MATERIALS AND METHODS

Urinary selenium level was determined fluorometrically, using 2,3-diaminonaphthalene (DAN), according to the method described in our previous paper (Hasunuma et al. 1982). Urine sample (0.2 mL) was mineralized with 1 mL of concentrated acid mixture (nitric, perchloric, and sulfuric acids, 2:1:0.1 by volume) by gentle refluxing on a sand bath for 2 hours. After cooling and addition of 6 M hydrochloric acid (0.3 mL), the digest was heated at 100^{o}C for 15 min. Following neutralization with 6 M sodium hydroxide (containing 0.1M EDTA), the pH was adjusted to about

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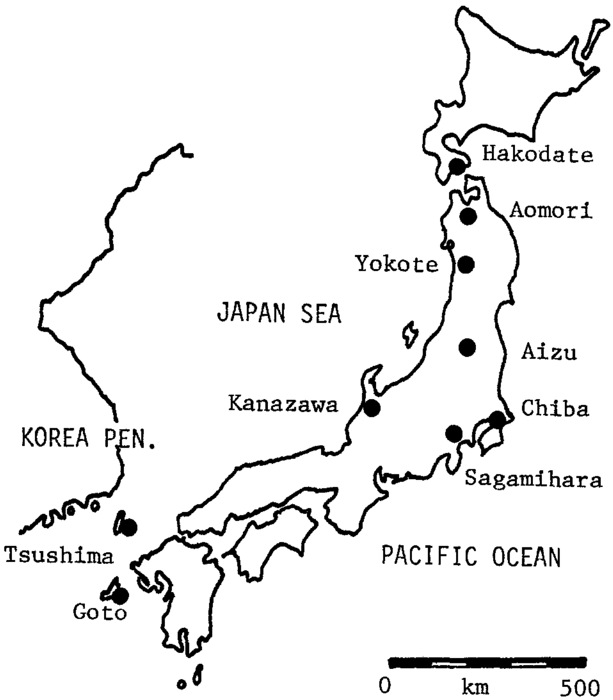

Figure 1. Sampling areas.

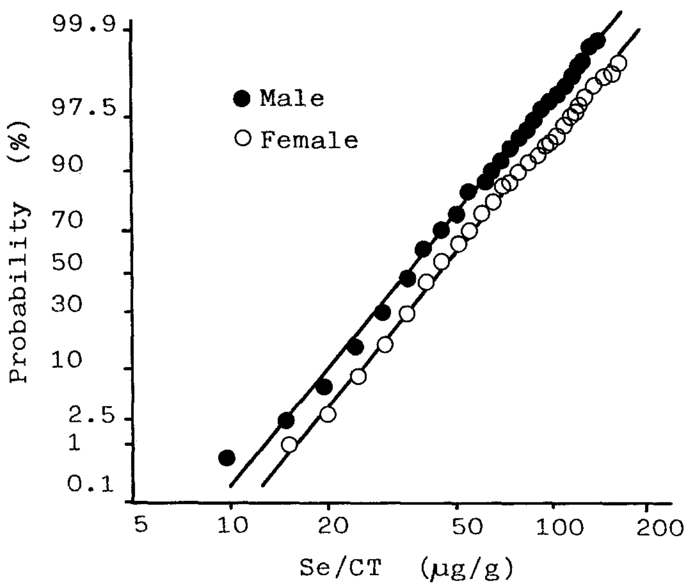

Figure 2. Probability plot of urinary selenium level.

1.5. On heating at 50^{0}C for 20 min after the addition of 0.3 mL of 0.1% DAN reagent, piazselenol was formed and extracted with 0.6 mL of cyclohexane. Fluorescence intensity of the extract was measured at 521 nm with excitation at 379 nm, using a Hitachi MPF-2A fluorospectrophotometer.

Urinary selenium level was represented as µg selenium/g creatinine (Se/CT) (Hojo 1982). Creatinine in the urine was determined by Folin-Wu method (Bosnes and Taussky 1945).

Single-void urine samples were collected from volunteers in Sagamihara area. The subjects consisted of healthy inhabitants (219 males and 208 females) and the outpatients of a clinic of general practice (212 males and 150 females), and were classified into 10 year-interval age classes. The subjects older than fifty-nine years old were classed in one group. Sample numbers of all classes are shown in Table 1.

We chose sampling areas as shown in Fig. 1, in order to include a wide variety of the locality: Hakodate; a fishery base city in Hokkaido: Aomori; a port city in the northern part of the Main Island: Yokote; a rural town of rice-farming area in the northern part of the Main Island: Aizu; a city in a basin near a volcano: Chiba and Sagamihara; satellite cities of Tokyo: Kanazawa; a city near the Japan Sea in the central part of the Main Island: Tsushima; a town in an island lying near Korea: Goto Islands; islands lying on the East China Sea. They are free from pollution with heavy metals except Aizu, where a factory had been leaking cadmium until some twenty years ago.

Samples collected in stoppered polymethylpentene tubes were overlaid with a few drops of toluene, and kept in a refrigerator until the determinations of selenium and creatinine. Transportation from distant places was carried out in the frozen state.

The minimally required number of samples in a class was estimated at 16, considering two-tailed t-test in which confidence interval, "specified difference" between Se/CT's, and standard deviation of Se/CT were 0.95, 5%, and 10%, respectively.

RESULTS AND DISCUSSION

For healthy volunteers in Sagamihara area, the frequency of Se/CT was plotted on a logarithmic normal probability paper for males and females separately. Results are shown in Fig. 2 suggesting that the frequency fits the logarithmic-normal distribution for both sexes. Therefore, we preferred geometric rather than arithmetic mean values in this study.

The urinary selenium level of the outpatients was compared with that of healthy inhabitants in the same area. No significant differences in variance and mean values of Se/CT could be found (Hasunuma et al. 1987). This means that medicines which have been administered routinely to the outpatients do not disturb the metabolic balance of selenium in general, thus allowing the outpatients to be included among healthy inhabitants in discussing the urinary selenium levels. Although certain diseases such as cancer and epilepsy (Hojo 1981), coelical disease (Fell et al. 1981) and burn (Hunt et al. 1983) are known to lower the urinary selenium levels, it might be the consequence of low selenium intake by hospitalization, for example, from total parenteral nutrition by tube feeding (Hunt et al. 1983).

Table 1. The distribution of urinary selenium levels among age and sex classes.

Age	Sex	No. of subjects	Se/CT (μg/g) Mean (S.D.) Geometric		Arithmetic	
0- 9	M	43	60.8	(1.5)	65.9	(26.5)
	F	34	57.5	(1.6)	64.8	(35.8)
10-19	M]***	160	42.4	(1.6)	46.6	(20.5)
	F	145	53.3	(1.6)	60.0	(32.1)
20-29	M	58	32.7	(1.4)	34.4	(10.1)
	F	40	33.4	(1.3)	34.8	(10.0)
30-39	M]***	56	33.0	(1.3)	34.1	(8.3)
	F	32	46.7	(1.5)	51.0	(22.2)
40-49	M]***	44	35.1	(1.3)	36.0	(9.9)
	F	44	48.3	(1.4)	51.4	(18.4)
50-59	M	37	34.8	(1.7)	39.1	(18.0)
	F	30	43.8	(1.6)	49.2	(26.2)
60-82	M	33	34.3	(1.4)	36.5	(12.7)
	F	33	40.2	(1.4)	41.1	(12.3)
Total	M]***	431	37.5	(1.5)	41.3	(19.3)
	F	358	45.7	(1.6)	51.3	(27.7)

M; male, F; female.
*** Significant differences are found between males and females in both cases ($p<0.001$).

Table 1 shows the distribution of mean urinary Se/CT values for both sexes among age classes. Higher Se/CT values were observed in the 0 to 9 year age class for both sexes ($p<0.05$). Lower creatinine level for the youngest age class seems to partially account for the result. It should be noted that Hausen et al. (1982) observed a similar trend in neopterine levels in human urine, and attributed it to the fast growth behavior of this age class.

Urinary selenium levels in female were higher than those in male, except in the 0-9 year age class. The sex-related differences were significant in the age classes 10-19, 30-39 and 40-49. Similar results were also obtained in other two districts, Aizu and Kanazawa (data not shown).

Although the use of creatinine level as an inner standard of urine concentration is widely accepted by many investigators, it may lead to some misunderstanding or confusion in comparing data from the subjects of different sex and age, because of differences in creatinine excretion. We found that selenium level can substitute creatinine as an inner standard in some cases. Urinary levels of some heavy metals were rather precise when represented as ratios to selenium level than to creatinine level (to be published).

To examine the locality, urinary selenium level of the age class of 20 to 29 years was compared among various areas, since this class showed little sexual difference and less standard deviation of Se/CT in Sagamihara. Mean urinary selenium levels of the subjects in the areas selected are shown in Table 2. It should be noted that significant differences between male and female were observed in some districts and in the total.

Among some of the districts, significantly different Se/CT values were observed. Chiba gave higher selenium level in female ($p<0.001$), while Yokote and Kanazawa showed lower Se/CT in male ($p<0.05$ and $p<0.01$). Mondragon et al.(1971) revealed that the higher levels of selenium in egg, milk, and vegetables in a

Table 2. Urinary selenium levels of the 20-29 age class in selected areas of Japan.

Area	Sex		No. of subjects	Se/CT(μg/g) Geometric mean	(S.D.)
Hakodate	M		15	34.4	(1.4)
	F		15	41.3	(1.5)
Aomori	M		20	34.8	(1.3)
	F		20	36.8	(1.3)
Yokote	M	]**	40	27.5	(1.5)
	F		40	34.5	(1.4)
Aizu	M	]*	20	34.8	(1.3)
	F		20	42.0	(1.4)
Chiba	M	]***	20	33.5	(1.4)
	F		20	49.0	(1.2)
Sagamihara	M		58	32.7	(1.4)
	F		40	33.4	(1.3)
Kanazawa	M	]***	20	26.6	(1.2)
	F		20	36.2	(1.4)
Tsushima	M		20	31.9	(1.4)
	F		19	35.9	(1.4)
Goto	M		20	31.2	(1.3)
	F		20	33.8	(1.4)
Total	M	]***	233	31.5	(1.4)
	F		214	37.1	(1.4)

Areas are shown in Fig. 1 and text.
M; male, F; female.
Significant differences are found between males and females: ***;($p<0.001$), **;($p<0.01$), and *;($p<0.05$).

district of Venezuela remarkably raised the urinary selenium level of the local school children. Results in the present study may also reflect some different environmental and/or social factors.

For the total samples investigated in this study (1164 male and 1036 female), the Se/CT values were 36.9 (S.D.=1.6) and 43.1 (S.D.=1.7) for male and female, respectively ($p<0.001$). These figures as well as the results shown in Tables 1 and 2, clearly show that the urinary selenium level in female is higher than in male, except in the 0-9 age class.

Some authors have found that selenium excretion is not affected by sex. Geahchan and Chambon (1980) have determined selenium levels for samples from 43 males and 33 females between 14 and 77 years old and presented the similar mean values, 11.7 and 11.8 µg Se/L urine, respectively. However, the number of samples might be too small to allow statistical criticism. Hongo et al. (1985) have concluded that sex-related difference of mean Se/CT (33 and 32, for male and female) is not significant. These values agreed with ours only in the 20-29 age class. We found that, however, differences of Se/CT values due to sex were significant even in this age class in four districts.

To conclude, urinary selenium level of Japanese (1164 males and 1036 females) were estimated as 36.9 µg Se/g creatinine for male and 43.1 µg Se/g creatinine for female, significantly higher than male. These values were higher in the 0-9 age class. Considerable differences in the values were observed among districts.

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