Tohoku J. Exp. Med., 2002, 197, 243-247

Short Report

Effects of Iron-Deficiency Anemia on Cadmium Uptake or Kidney Dysfunction Are Essentially Nil among Women in General Population in Japan

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TSUKAHARA, T., EZAKI, T., MORIGUCHI, J., FURUKI, K., UKAI, H., OKAMOTO, S., SAKURAI, H. and IKEDA, M. *Effects of Iron-Deficiency Anemia on Cadmium Uptake or Kidney Dysfunction Are Essentially Nil among Women in General Population in Japan.* Tohoku J. Exp. Med., 2002, **197** (4), 243-247 —— In the present study, 1476 adult women in 6 prefectures in Japan volunteered to offer peripheral blood and spot urine samples, and to complete questionnaires on social habits and health. Blood samples were analyzed for iron, ferritin and TIBC in serum in addition to RBC, Hb and Cd in whole blood. Urine samples were analyzed for Cd, α_1-MG, and β_2-MG; the measures were corrected for creatinine and were expressed as e.g., Cd-Ucr. Among 1212 never-smokers, 37 women with <25 ng ferritin/ml serum and <10 g Hb/100 ml blood were classified as the anemics, whereas 701 women with $\geqq 25$ ng/ml ferritin and $\geqq 10$ g/100 ml Hb were taken as controls. Matching by age and the prefecture of residence was successful for 34 anemics. Comparison (by paired t-test) of Cd in blood, and Cd, α_1-MG and β_2-MG in urine (as corrected for creatinine) of the anemics with that of matched controls showed no significant differences. Thus, it appeared likely that the current level of iron insufficiency among general women population in Japan may not induce substantial increase in Cd absorption or Cd-associated kidney dysfunction. ———— anemia; cadmium; iron deficiency; microglobulins in urine; women

Received May 13, 2002; revision accepted for publication August 14, 2002.
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It is known in animal experiments that metabolic interaction may take place between cadmium (Cd) and iron (e.g., Goyer 1997). In humans, Flanagan et al. (1978) reported a reverse relation of the rate of dietary Cd absorption with serum ferritin level. Berglund et al. (1994) observed a close correlation of Cd in blood with serum ferritin levels. Intake of iron is often insufficient among general population, particularly women, in Japan (e.g., Shimbo et al. 1996), where dietary Cd intake is known to be high (e.g., 30–40 μg/day), despite substantial reduction during recent years (Ikeda et al. 2000; Watanabe et al. 2000). Thus, a possible increase of Cd absorption due to iron-deficiency anemia is a potential risk in public health in Japan.

SUBJECTS AND METHODS

The survey was carried out in 6 institutions, one each in 6 prefectures in Japan in 2002, after the approval of the protocol by the Ethics Committee of Kyoto Industrial Health Association. In total, 1476 women (aged 20–74 years, with no occupational exposure to Cd) provided informed consents to participate in the study, to offer blood and spot urine samples, and to complete questionnaires on current health status, clinical history and social habits. Each subject was given a registration number according to the location of the institution (from north to south) and the order of her visit to the institution. Those who were either pregnant, lactating or under clinical treatment, or had history of kidney diseases, were excluded from the study. Both current and past smokers were also excluded, so that a group of 1212 never smoking women was available as a population for statistical analysis.

Blood samples were subjected to hematological and serum-biochemical examinations including red blood cell counting (RBC), and determination of hemoglobin (Hb), iron (by the Nitroso PSAP method), total iron binding capacity (TIBC; the same as iron), and ferritin (by PRHA) in serum and Cd in whole blood (Cd-B; by the graphite furnace AA). Urine samples were employed for determination of Cd (Cd-U; the same as Cd-B), α_1-microglobulin (α_1-MG; by RIA), β_2-microglobulin (β_2-MG; by RIA), and creatinine (cr; by the alkali-picric acid method); the pH of urine samples for β_2-MG measurement was brought to 6–8 immediately after sample collection. The measures in urine after correction for creatinine (Jackson 1966) were expressed as Cd-Ucr, α_1-MG-Ucr and β_2-MG-Ucr. Analyses were made in a single laboratory except for RBC and Hb, which were made locally.

Distribution of Cd-B, Cd-Ucr, α_1-MG-Ucr and β_2-MG-Ucr was assumed to be log-normal (Ikeda et al. 2000), whereas a normal distribution was assumed for age, and hematological/serum-biochemical parameters, so that the distribution was expressed in terms of a geometric mean (GM) and a geometric standard deviation (GSD) in the former case, and of an arithmetic mean (AM) and an arithmetic standard deviation (ASD) in the latter. In calculating GM and GSD, the values for <DL cases were assumed as if they had been half the detection limit. Statistical significance of the difference was examined by paired t-test.

RESULTS AND DISCUSSION

With ferritin (the cut-off value; 25 ng/ml) and Hb concentration (10 g/100 ml) as classification criteria, the 1212 never-smoking women were classified into three groups of 37 anemics with low ferritin and low Hb, 701 normal controls with sufficient ferritin and Hb, and remaining 474 women with low ferritin but sufficient Hb. There was no case whose ferritin was sufficient but Hb was reduced.

An age- and prefecture-matched control was selected for each of anemic subjects; prior-

ity was given in accordance with the registration number so that an anemics with a younger number was matched with a control with a younger number in case more than one case of either anemics or controls were available at the same age and in the same prefecture. In practice, 34 anemic women were matched with controls; it was not possible to find controls for three anemic cases.

Comparison (by paired t-test) between the cases and the controls in terms of the six blood/serum and the three urinary parameters (Table 1) showed that iron, Hb and RBC were lower ($p<0.01$) and TIBC was higher ($p<0.01$) in the anemic women than in the matched controls as expected. Differences in Cd-B, Cd-Ucr, α_1-MG-Ucr and β_2-MG-Ucr were however statistically insignificant ($p>0.05$), although GM values for Cd-B, Cd-Ucr and β_2-MG-Ucr tended to be greater for anemics (3.1 μg/liter, 1.3 μg/g cr, and 133 μg/g cr, respectively) than for the controls (2.6 μg/liter, 1.1 μg/g cr, and 112 μg/g cr). The reverse was the case for α_1-MG-Ucr, i.e., 2.42 μg/g cr for the anemics and 2.66 μg/g cr for the controls.

Literature survey shows that retention of Cd was increased after experimental depletion of Fe supply to rats (e.g., Schaefer et al. 1990). Cd administration to rats reversely reduced Fe contents in the intestine (e.g., Sugawara and Sugawara 1991). In humans, Flanagan et al. (1978) observed that the dietary Cd absorption was higher (8.9%) among the subjects with low ferritin in serum (i.e., <20 ng/ml) than the rate (2.3%) among those with normal ferritin levels (>23 ng/ml). In a group of nonsmoking women, Berglund et al. (1994) observed close correlations of Cd-B with serum ferritin and

TABLE 1. *Comparison between anemic and iron-deficient groups with normal controls*

Matrix Item	Unit	Anemics[a]				Paired controls[b]				p[f]
		Mean[e]	S.D.[e]	Min	Max	Mean[e]	S.D.[e]	Min	Max	
Age	years	44.3	5.4	29	54	44.3	5.4	29	54	ns
Blood/serum										
Ferritin	ng/ml	<25[g]				50.7	65.6	25	400	
Iron	μg/100 ml	18.1	9.5	9	60	109.1	32.0	31	169	↓
TIBC	μg/100 ml	427.9	44.0	325	510	344.8	36.0	263	422	↑
Hb[b]	g/100 ml	8.8	0.8	6.6	9.9	13.4	0.8	11.9	15.2	↓
RBC	10^4/mm^3	408.6	36.3	350	484	435.2	25.0	376	477	↓
Cd	μg/l	3.1	2.11	0.6	17.4	2.6	2.66	<DL[g]	17.8	ns
Urine (corrected for creatinine)										
Cd	μg/g cr	1.27	1.87	<DL[g]	5.4	1.09	2.14	<DL[g]	4.0	ns
α_1-MG	mg/g cr	2.42	2.14	<DL[g]	7.2	2.66	2.26	<DL[g]	16.7	ns
β_2-MG	μg/g cr	133.0	1.57	58	393	111.8	1.70	19	231	ns

[a]Anemics with <25 ng/ml ferritin and <10 g/100 ml Hb.
[b]Controls with ≧25 ng/ml ferritin and ≧10 g/100 ml Hb.
[c]Fe-deficients with <25 ml ferritin but ≧10 g/100 ml Hb.
[d]Controls with ≧25 ng/ml ferritin and ≧10 g/100 ml Hb.
[e]AM and ASD for parameters in blood (except for Cd-B) or serum, and GM and GSD for Cd-B and parameters in urine.
[f]A p-value for the difference from the paired control group as examined by paired t-test ($n=34$); the direction of arrows show that the value for the anemics is higher or lower than that for the controls (an arrow for $p<0.01$, and ns for $p>0.05$).
[g]The detection limit (DL) is 0.5 μg/l for Cd-B and Cd-U, 0.6 μg/l for α_1-MG and 25 ng/ml for ferritin.

dietary intake of fiber; Cd in 24-hour urine samples also correlated with ferritin and fiber, but the statistical power was weaker.

In the present study, elevation in Cd-B and Cd-Ucr levels in the anemic women was statistically insignificant although GM values tended to be greater, despite the fact that ferritin levels were clearly lower than normal (Table 1), and in that sense the observation agrees with the findings of Flanagan et al. (1978) and Berglund et al. (1994) only partly. The lack of significant increase in Cd-Ucr in the anemic women suggests that the extent of Fe deficiency among the population studied may not be severe enough to induce a substantial increase in Cd absorption. Indeed, Cd interaction with iron metabolism in rats was dose-dependent, e.g., being positive at the Cd dose of 50 to 100 μg/ml or above in drinking water (estimated to be 4.5 to 9 mg/kg b.w./day) (Chmielnicka and Sowa 1996). The estimated Cd intake among general women population in Japan is 30-40 μg/day (Ikeda et al. 2000; Watanabe et al. 2000) or 0.5-0.7 μg/kg b.w./day when an average body weight of 60 kg is assumed for women. This dose is less than one 10^3 th of the dose (5 to 10 mg/kg b.w./day) used in the rat experiment (Chmielnicka and Sowa 1996).

In overall evaluation, therefore, it appears likely that the current prevalence of iron-deficiency may not induce a substantial increase in Cd absorption or urinary microglobulins among general women population in Japan. Although the PRHA method for the determination of serum ferritin is sensitive enough to identify iron-deficient subjects, its semi-quantitative nature does not allow dose-effect analysis in correlation of serum ferritin with Cd-B or Cd-U. Life-style information was inevitably depended on self-administered questionnaires and it was not possible to confirm, e.g., by personal interview. Furthermore, subjects scattered in 6 prefectures, and no reliable quantitative information was available on dietary intakes of cadmium, iron and other elements of toxicological or nutritional importance.

Serum ferritin is currently under re-examination by more quantitative CLIA. The results will be reported in combination with the observation in women who have sufficient Hb but reduced ferritin.

Acknowledgements

A part of this work was supported by research grants from the Ministry of Health, Labor and Welfare, for the fiscal years 2000-1, and from the Ministry of Education, Science and Technology for the fiscal year 2001, the Government of Japan. The authors are grateful to Miss F. Ohashi for her skilful work in statistical analysis. Support of the following six organizations are gratefully acknowledged; Miyagi Occupational Hygiene and Medical Association (Sendai), Kanagawa Health Service Association (Yokohama), Niigata Association of Labor Health (Niigata), Nishinihon Occupational Health Service Center (Kitakyushu), Okinawa General Health Service Association (Naha), and Kyoto Industrial Health Association (Kyoto), Japan.

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