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Comparison of Hair Mercury Concentrations between Married Couples

Weishi CHEN[1], Junko MAGARA[2], Kazuo ENDOH[2], Fumio SHIRAI[3] and Masaharu YAMAMOTO[2]

[1]Department of Hygiene and Preventive Medicine, Niigata University School of Medicine, Japan, and Health and Anti-Epidemic Station of Guangdong Province, Guangzhou, People's Republic of China, [2]Department of Hygiene and Preventive Medicine, Niigata University School of Medicine, [3]Niigata Prefectural Research Laboratory for Health and Environment

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Summary. **Hair samples of 53 couples in Niigata City, Japan were analyzed for total-Hg by the cold atomic absorption method and methyl-Hg by the gas chromatographic method. The concentrations of hair total-Hg and methyl-Hg were 4.01±1.736 μg/g (mean±S.D.) and 3.04±1.401 in husbands; 1.99±0.968 and 1.54±0.687 in wives. It was found that the concentrations of total-Hg and methyl-Hg in the hair of the husbands were significantly higher than those of their wives ($p<0.01$, respectively). The correlations of hair mercury concentrations between couples were found ($r=0.420$, $p<0.01$ for total-Hg; $r=0.484$, $p<0.01$ for methyl-Hg). The concentration of mercury in the hair of couples with high fish intake frequency was higher than that with a low one. It is suggested the fish intake might have a predominant effect on the concentration of mercury in the hair.**

INTRODUCTION

Human scalp hair has been proved a practical indicator for metallic environmental pollutants and is widely used in epidemiological studies on individuals who are suspected of being exposed to mercury from nonoccupational environments.[1-4] It has been reported that hair mercury concentration is significant in reflecting the total body burden of mercury and that hair mercury concentration is related to the consumption of fish.[5-9] Fish is a major component of the Japanese diet. It has also been reported that hair mercury contents are higher in men than in women,[10] and might also be influenced by lifestyles within a family.[11] Therefore, it seems worthwhile to investigate mercury contents in the hair of couples.

In this study, the concentration of total-Hg and methyl-Hg in scalp hair of couples living in Niigata City, Japan, were analyzed and compared, and the factors that might affect the amount of mercury in hair were studied and discussed.

MATERIALS AND METHODS

Fifty three married couples having lived in Niigata City over three years but not living in the area along the Agano River, Niigata Prefecture, where the second outbreak of Minamata disease occured, were chosen as the study subjects. The average ages were 43.2 years for husbands and 40.7 for wives. Polyethylene bags for hair samples and questionnaires were sent to them. When the subject underwent his/her regular haircut, about 4-5 g hair was collected. At the same time, a questionnaire was filled out individually, concerning the name, sex (husband or wife), age, address, job, recent consumption frequencies (daily or weekly) of fish (including shellfish) and other meat (such as chicken, pork, beef, etc), and hair treatment such as permanent waves. All hair samples and questionnaires were collected from June to Semptember, 1988. There were 33 wives, but no husbands, who had received permanent waves.

The hair samples were washed with a mixture of ethanol and acetone(1:1; both were Extra Pure Reagent from Wako Pure Chemical Industries Ltd., Osaka) 3 times, lightly disturbed for 10 min at every washing, dried in the air, cut into 3-5 mm in length with stainless steel scissors, put into a clear polyethylene bag, and stored in a desiccator till analysis.

Total-Hg in hair was analyzed by the cold atomic absorption method[12] and methyl-Hg, by the gas

chromatographic method,[13,14] both with modifications which have been published elsewhere.[15]

Student's or Welch's t test was used for statistical analysis of data. Mercury concentrations were expressed in terms of mean±S.D.

RESULTS

The age distributions and concentrations of hair total-Hg and methyl-Hg of the 53 couples are shown in Table 1. The average concentrations of total-Hg and methyl-Hg in the hair of the husbands were found to be significantly higher than those in the hair of the wives ($p<0.01$, respectively). Significant differences in hair mercury concentration between couples were found in most of the age groups. The mean contents of methyl-Hg in hair were 75.8% of total-Hg in the husbands and 77.4% in the wives, respectively.

Fig. 1 shows the scatter diagrams and the regression lines of total-Hg and methyl-Hg concentrations in the hair among the 53 couples. Significant correlations of hair mercury concentrations between husbands and wives were found ($r=0.420$, $p<0.01$ for total-Hg and $r=0.484$, $p<0.01$ for methyl-Hg). No significant correlation between hair mercury contents and the age of the couple was found.

Table 2 shows the comparison of the average hair mercury concentrations between wives with and without permanent waves. The average total-Hg concentration in the hair of the 33 wives undergoing beauty treatment for permanent waves was lower than that in the hair of the 20 wives without permanent waves ($p<0.05$). However, no significant dif-

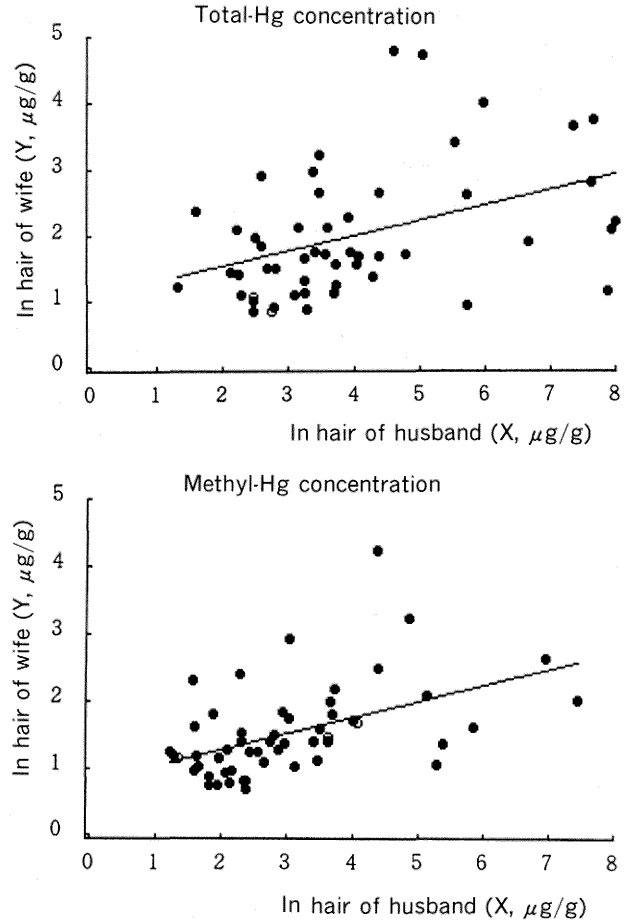

Fig. 1. Scatter diagrams relating total-Hg and methyl-Hg concentrations in the hair between husbands and wives, with regression lines (Total-Hg: $Y=0.234X+1.056$, $n=53$, $r=0.420$, $p<0.01$; Methyl-Hg: $Y=0.237X+0.818$, $n=53$, $r=0.484$, $p<0.01$).

Table 1. Concentration of mercury in the hair of husbands and wives

Age group	Husbands			Wives		
	N†	Total-Hg	Methyl-Hg	N†	Total-Hg	Methyl-Hg
		(Mean±S. D., μg/g)			(Mean±S. D., μg/g)	
~29	1	2.58	1.60	3	2.42±1.114	1.69±0.678
30~39	21	3.60±1.293 **	2.63±1.167 **	27	1.74±0.841	1.37±0.550
40~49	22	4.61±2.215 **	3.53±1.665 **	14	2.26±1.083	1.65±0.708
50~59	6	3.88±0.843 *	3.32±0.702 *	6	2.41±1.242	2.00±1.159
60~	3	3.14±0.676 *	2.25±0.602 N.S.	3	1.71±0.372	1.51±0.335
Total	53	4.01±1.736 **	3.04±1.401 **	53	1.99±0.968	1.54±0.687
Range		1.32~8.00	1.22~7.43		0.83~4.79	0.68~4.26

† The number of husbands or wives. *$p<0.05$, **$p<0.01$, N.S.: not significant between husbands and wives compared with the same form of mercury in the same age group.

ference in hair methyl-Hg between wives with and without permanent waves was found.

Table 3 shows the result of comparisons of hair mercury concentrations between the couples with different amount of fish consumption and permanent waves. In Group A, where the fish intake frequencies were identical between the husbands and wives, the concentrations of total-Hg and methyl-Hg in the hair of the couples with high fish intake frequency (7 meals or more/week) were higher than those in the hair of couples with a low one (6 meals or less/week); the concentrations of total-Hg and methyl-Hg in the hair of the husbands were significantly higher than those in the hair of the wives in cases of the same fish intake frequency. In Group B, where the wives were without permanent waves, only the average concentration of total-Hg in the hair of the wives with high fish intake frequency was significantly greater than that in the hair of the wives with a low one ($p<0.05$); except for the total-Hg concentration in the hair of the couples with high fish intake frequency, the total-Hg and methyl-Hg concentrations in the hair of the

Table 2. Comparison of concentration of mercury in the hair of wives with or without permanent wave beauty treatment

Permanent wave	N †	Total-Hg (Mean±S.D., μg/g)		Methyl-Hg (Mean±S.D., μg/g)	
+	33	1.78±0.954	*	1.45±0.713	N.S.
−	20	2.34±0.910		1.71±0.648	

† The number of wives.
*$p<0.05$, N.S.: not significant.

Table 3. Comparisons of hair mercury concentration between husbands and wives grouped by the factors of fish intake frequency and permanent waves

Group	Fish intake frequency (meal/week)	Number of couples	Total-Hg (Mean±S,D., μg/g) Husbands		Total-Hg (Mean±S,D., μg/g) Wives		Methyl-Hg (Mean±S.D., μg/g) Husbands		Methyl-Hg (Mean±S.D., μg/g) Wives	
A	≦ 6	37	3.43±1.077≠	**	1.67±0.604	**	2.61±0.993≠	*	1.38±0.157	**
	≧ 7	13	5.15±2.161≠		3.00±1.152		3.95±1.783≠		2.09±0.878	
	Total	50	3.88±1.605≠		2.02±0.968		2.96±1.362≠		1.57±0.696	
B	≦ 6	13	3.51±1.510≠	N.S.	1.97±0.709	*	2.69±1.479 †	N.S.	1.63±0.681	N.S.
	≧ 7	6	5.23±2.356	N.S.	3.06±0.952		4.01±1.972 †		1.92±0.626	
	Total	19	4.05±1.932≠		2.31±0.928		3.11±1.714≠		1.72±0.661	

Group A: 50 couples from 53 couples based on the identical fish intake frequency. Group B: 19 couples without permanent waves were from the 50 couples of Group A. *$p<0.05$, **$p<0.01$, N.S.: not significant. †$p<0.05$, ≠$p<0.01$ between husbands and wives with the same fish intake frequency.

Table 4. The results of partial correlation coefficients of mercury concentrations in the hair of husbands as well as wives

Factor	Number of categories	Husbands (N=53) Total-Hg	Husbands (N=53) Methyl-Hg	Wives (N=53) Total-Hg	Wives (N=53) Methyl-Hg
Age	5	0.403**	0.374**	0.241 N.S.	0.290*
Fish†	2	0.660**	0.566**	0.563**	0.405**
Meat††	2	0.486**	0.342**	0.078 N.S.	0.033 N.S.
Perm‡	2			0.315*	0.186 N.S.
R^2§		0.475**	0.376**	0.454**	0.302**

†Fish intake frequency. ††Other meat intake frequency. ‡Hair permanent waves in wives.
§ Square of multiple correlation coefficient. *$p<0.05$, **$p<0.01$, N.S.: not significant.

husbands were significantly higher than those in the hair of the wives.

Significant differences in the hair mercury concentrations between the couples with high and low intake frequencies of other meat were not found.

Table 4 shows the results of partial correlation coefficients of the total-Hg and methyl-Hg concentrations in the hair of the 53 husbands as well as the 53 wives to factors of age (5 categories consisting of 5 age groups. See Table 1), fish and other meat intake frequencies (2 categories in each, i.e., 6 meals or less/week and 7 meals or more/week), permanent waves (2 categories, i.e., "+" and "−" for wives), and the multiple correlation coefficients by the multiple regression analysis method (Hayashi quantification theory I[16]) to identify the significance and importance of influencing factors on mercury in the hair of the couples. The orders of significant factors for the husbands were "Fish, Meat and Age" in hair total-Hg and "Fish, Age and Meat" in methyl-Hg; and those for the wives, were "Fish and Permanent waves" in total-Hg and "Fish and Age" in methyl-Hg.

DISCUSSION

The factors which may influence the amount of mercury in hair could be considered as endogenous and exogenous sources. The contents of hair mercury which reflect the body burden come from an endogenous source, i.e., through intaking food, water, air, etc., in which the food, especially fish and shellfish contaminated with mercury, has been noted as one important factor influencing the content of mercury in hair from nonoccupational exposure to mercury.[1,5,7]

In Papua New Guinea,[5] the hair methyl-Hg content was as high as 15.5 μg/g in a population who consumed fish 2-3 times daily. Nishima et al.[17] found that the total-Hg and methyl-Hg levels in hair and blood were significantly higher in fish eaters than the controls. Tsugane[11] reported that dose-response relationships between the hair mercury content and fish consumption were found among Japanese immigrants in South America. In the study by Ando,[18] the organic-Hg concentration in the hair of husbands, but not wives, was found to increase steadily with the amount of fish consumption. In the present study, the concentrations of total-Hg and methyl-Hg in the hair of the couples with high fish intake frequency were found to be higher than those of the couples with a low one. It is suggested that the fish intake may have a predominant effect on the amount of mercury in the hair.

Occupational exposure to mercury can make the hair mercury content higher than that of non-exposed individuals. All subjects of this study had neither a history of occupational mercury exposure nor any other source of environmental mercurial pollutants in the neighborhood, such as factories, mines or refineries. It could be considered that the hair mercury of the couples was from nonoccupational exposure.

Beauty treatment for hair, such as permanent waving and dyeing, can change the quality of hair, and was noted as an important exogenous factor influencing the contents of elements in the hair.[19] It has been reported that the contents of organic-Hg in the hair of the women with permanent waves were lower than those of the women without permanent waves and that the treatment for hair dyeing could increase the contents of mercury in the hair.[18] In this study, an influence of permanent waving on hair total-Hg was found. However, the conspicuous impact of hair dyeing on mercury contents could not be determined because only one subject (wife) had her hair dyed, and the concentrations of total-Hg and methyl-Hg in her hair were 2.27 and 1.54 μg/g, close to or within the average levels for all wives, respectively.

The finding of significant correlations of hair mercury concentrations between couples suggested that their common lifestyle influenced the concentration of mercury in their hair.

In this study, the concentration of mercury in the hair of the husbands was found to be higher than that in the hair of the wives. This could partly be influenced by the permanent waving and lower fish intake frequency. However, after excluding the factors of permanent waving and fish intake frequency, the methyl-Hg concentration in the hair of the husbands was still higher than that of the wives even in cases of the same high fish intake frequency (see Table 3). Generally speaking, the amount of fish consumed by husbands may be more than that by wives, even with the same fish intake frequency. The results of multiple regression analysis show that the other meat intake frequency and age were also significant in influencing hair mercury contents. This suggests that other factors can not be overlooked.

It is reported that fish and shellfish contribute 50% of the amount of mercury in the Japanese diet.[20] Unfortunately, detailed data on the amount of fish consumed are unavailable. Other factors, such as menstruation, pregnancy, delivery and breast feeding[21-23] and the different amount of hormones may also influence the hair mercury contents of

women. The disputable factors may be ethanol and smoking. It is reported that ethanol administration increased the level of methyl-Hg in tissues and organs of rats.[24] However, the effect of ethanol has not been found in human hair.[18] In regard to the effect of smoking, there have been arguments about whether it contributes to increasing mercury amounts in human hair.[18,25] All of these still remain to be studied in future.

The average hair total-Hg amounts for Japanese were reported to be 4.1 ppm for men and 2.3 for women in Sapporo,[26] 5.26 ppm and 2.97 in Tokyo,[25] 11.18 ppm and 6.74 in Kagoshima,[27] respectively. In this article the amounts of hair mercury of the husbands (men) and wives (women) were within the levels of the Japanese as mentioned above (see Table 1).

The second outbreak of Minamata disease, methyl-Hg poisoning, occurred in Niigata during 1964-65, similar to the first one that occurred at Minamata Bay during 1953-60, and has been ascribed to the ingestion of fish and crustaceans contaminated with methyl-Hg.[5,28] The onset of the symptoms of mercury poisoning may occur at total-Hg levels as low as 50 μg/g in hair and 0.2 μg/g in whole blood.[5] It is reported that the contents of total-Hg in hair of patients of Minamata disease in Niigata were in the range of 104-425 ppm.[29] The contents of total-Hg in the hair of the couples of this study were far lower than the levels of mercury poisoning.

Different methods of washing hair samples can also influence the contents of trace elements in hair.[30-32] There are many methods of washing hair samples for the analysis of mercury: with detergent and distilled water;[32] acetone and water (1:1);[6] ionic detergent followed by successive rinses with water, acetone, and ether;[33] successively with enough aceton, ether, and acetone;[34] or EDTA solution and deionized water.[11] In the present study, ethanol and accetone were used. The method was recommended by Kozuka et al.[30] as it can be performed easily and has a good reproducibility. It is reported that the acetone could not methylate mercury,[6] and there was nearly no loss of bromine, mercury, zinc, or copper in the hair by the treatment of a mixture of ethanol and acetone.[30]

Among the factors mentioned above, it is concluded from the present experiment that the fish intake frequency is a predominant factor on influencing the concentration of mercury in the hair.

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