### Short Communication

# Effects of Hair Treatment on Hair Mercury—The Best Biomarker of Methylmercury Exposure?

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## Abstract

Objectives: Exposure misclassification is a major obstacle to obtain accurate dose-response relationships. In order to solve this problem, the impact of hair treatment on total mercury in hair was assessed in Japanese women.

Methods: A cross-sectional study was carried out among 327 women at age 24–49 years to determine hair mercury levels and estimate daily mercury intakes from seafood by using a food frequency questionnaire.

Results: Hair mercury levels in the women and daily mercury intake ranged from 0.11 to 6.86 (median 1.63)  $\mu$ g/g and from 0.77 to 144.9 (median 15.0)  $\mu$ g/day, respectively. The hair mercury was positively correlated with the daily mercury intake (p<0.001). When the women were divided into two subgroups based on artificial hair-waving, hair coloring/dyeing, residence (non-fishing and fishing areas), and working status, a significant difference in the hair mercury level was observed between the women with and without artificial hair-waving only (p<0.001). The multiple regression analysis showed that the log-transformed hair mercury level was significantly related to the log-transformed daily mercury intake (standardized regression coefficient  $\beta$ s=0.307) and artificial hair-waving ( $\beta$ s=-0.276); but not to hair coloring/dyeing, residence, working status or age. Permanent hair treatment was estimated to reduce total mercury in hair by approximately 30%, after adjusting for daily mercury intake and other possible factors.

Conclusions: These findings suggest that hair mercury is not the best biomarker of methylmercury exposure when a study population includes women with artificial hair-waving.

Key words: hair mercury, daily mercury intake, permanent hair treatment, exposure biomarker, Japanese women

## Introduction

The total mercury concentration in hair has been reported to be affected by various preanalytical factors besides analytical imprecision, for instance, adhesion of environmental mercury vapor (1), permanent hair treatment (2–4), and hair color (5), although hair mercury is believed to reflect the average methylmercury concentrations circulating in the blood (6, 7)

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and it is frequently used as the biomarker of individual exposures to methylmercury. If any preanalytical factors exist in a study population, a dose response (or effect) relationship obtained from a study based on hair mercury may be overlooked or underestimated (8), because the effects of such factors on hair mercury do not seem to have been explored in detail. In fact, neither the Faroese birth cohort study nor the Seychelles child development study provided information on hair treatment in the Materials and Methods sections (9–11). The degree of exposure misclassification in hair mercury may be inferred from comparisons between exposure indicators. In this study, we determined hair mercury levels in Japanese mothers and estimated daily mercury intakes from seafood by using a food frequency questionnaire (FFQ), in order to evaluate the effects of preanalytical factors, especially hair

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treatment of artificial waving and coloring/dyeing, on hair mercury.

## **Materials and Methods**

## Subjects

The nature of the procedures used in this study was explained to parents with a first grader (7-year-old child), of 28 different elementary schools in Akita and Tottori Prefectures, Japan, 14 of which were located near a fishing harbor (i.e., fishing areas). In Japan, there were many mines and smelters 30 years ago, and it is probable that soil or water was contaminated by lead, cadmium, mercury vapor, etc; therefore, the study population did not include people who came from such areas. Finally, 327 mothers participated (12). This study was carried out with the approval of the ethical review committee at the Akita University School of Medicine.

#### Methods

Hair samples were collected by cutting strands of hair close to the scalp from the occipital area in all mothers. The hair length ranged from 5 to 30 (mean 10) cm. Total mercury in aliquots of dried hair samples (15 to 20 mg), which were cut into small pieces (<2 mm) with scissors after being washed well with detergent and rinsed two times with acetone, was determined by the cold vapor atomic absorption spectrophotometry method at the National Institute for Minamata Disease (13, 14).

A detailed survey of the frequency and volume of seafood ingested in a year was conducted by a trained interviewer at the schools or civic centers, showing 25 kinds of full-scale pictures including fish, shellfish and seaweed items (e.g., tuna, swordfish, skipjack tuna, codfish, flatfish, mackerel, sardine, sea bream, whale, salmon, eel, crab, prawn, octopus, squid, oyster, sea urchin, fish paste, shellfish, seaweed, etc.) to each mother, based upon the FFQ (4, 15), i.e., a modified version of Date et al. (16). Then, the total mercury intake from seafood ( $\mu$ g/year) was estimated on the basis of the previous references on mercury concentrations in seafood (17, 18), and daily mercury intake (µg/day) was calculated dividing by 365 days. Moreover, questionnaires on artificial hair-waving and hair coloring/ dyeing were collected from the mothers, and a medical doctor confirmed them, together with working status, using the interview method.

## Statistical analysis

The relationships between the hair mercury level and daily mercury intake were analyzed by the Spearman rank correlation coefficient ( $r_s$ ). The Mann-Whitney U test was used to compare the two subgroups divided on the basis of artificial hair-waving, hair coloring/dyeing, residence, and working status. Logarithmic transformation ( $\log_{10}$ ) of the hair mercury concentration and daily mercury intake was used because of skewed distributions. The relation of the daily mercury intake, artificial hair-waving, hair coloring/dyeing, residence, working status, and age to the hair mercury level was examined by the multiple regression analysis. Artificial hair-waving, hair coloring/dyeing, and working status were scored as "absence"=0 and "presence"=1; also, residence was scored as "non-fishing area"=0 and "fishing area"=1. Also, the analysis of covariance was used to compare hair mercury concentrations in mothers with and without artificial hair-waving (or hair coloring/dyeing) after adjustment for daily mercury intake, residence, working status, age, and hair coloring/dyeing (or artificial hair-waving). All analyses, with two-sided p values, were performed using the Statistical Package for the Biosciences (19).

## Results

The hair mercury concentrations in the 327 Japanese mothers at 24–49 (mean 36) years of age ranged from 0.11 to 6.86 (median 1.63)  $\mu$ g/g, and the daily mercury intakes, calculated from the FFQ data, were between 0.77 and 144.9 (median 15.0)  $\mu$ g/day. Among the mothers, there was a significant correlation between the hair mercury and daily mercury intake (Fig. 1). As the average value of body weight was 54.6 kg in 16,353 women aged 30–44 years, residing in Akita Prefecture (2002's data of the Akita Prefectural Center of Health Care), body weight of 55 kg for mothers was used to convert daily ingested dose ( $\mu$ g/day) to that per body weight ( $\mu$ g/kg body weight per day). Assuming the methylmercury content of 93% in seafood mercury (20), the mothers were suspected of having ingested methylmercury at a geometric mean of 0.25  $\mu$ g/kg body weight per day, as shown in Table 1.

When the 327 mothers were divided into two subgroups based on artificial hair-waving, hair coloring/dyeing, residence, and working status (Table 2), there was only a significant differ-



Fig. 1 Relationship between daily mercury intake and hair mercury level in 327 Japanese mothers.  $r_s$ , open circle, and closed circle indicate the Spearman rank correlation coefficient, and mothers with and without artificial hair-waving, respectively.

Table 1 Distribution of daily methylmercury intakes (µg/kg body
weight per day), estimated from 327 Japanese mothers, under the
assumption that body weight of mother was 55 kg, and methylmer-
cury-mercury ratio in seafood was 0.93

Daily intake	Number of mothers	Proportion (%)
≤0.1	27	8.3
≤0.2	89	27.2
≤0.3	86	26.3
≤0.4	60	18.3
≤0.5	27	8.3
>0.5	38	11.6

	Median (range)	Median (range)	p*
Artificial hair-waving:	Absence (N=219)	Presence (N=108)	
Hair mercury (µg/g)	1.81 (0.39~5.83)	1.31 (0.11~6.86)	< 0.0001
Daily mercury intake (µg/day)	14.5 (1.61~94.1)	16.5 (0.77~144.9)	0.11
Hair coloring/dyeing:	Absence (N=69)	Presence (N=258)	
Hair mercury (µg/g)	1.74 (0.64~6.86)	1.58 (0.11~5.83)	0.08
Daily mercury intake (µg/day)	16.3 (2.65~74.5)	14.7 (0.77~144.9)	0.68
Residence:	Non-fishing areas (N=127)	Fishing areas (N=200)	
Hair mercury (µg/g)	1.84 (0.48~4.79)	1.55 (0.11~6.86)	0.08
Daily mercury intake (µg/day)	15.0 (0.77~74.5)	16.6 (1.63~144.9)	0.94
Working status:	Without job (N=120)	With job (N=207)	
Hair mercury (µg/g)	1.64 (0.42~4.86)	1.62 (0.11~6.86)	0.63
Daily mercury intake (µg/day)	14.9 (2.80~144.9)	15.0 (0.77~94.1)	0.83

Table 2 Mercury in hair and daily mercury intake in two subgroups divided according to artificial hair-waving, hair coloring/dyeing, residence, and working status in Japanese mothers

\* Mann-Whitney U test.



Fig. 2 Hair mercury concentrations in 219 mothers without and 108 mothers with artificial hair-waving after the adjustment for daily mercury intake, hair coloring/dyeing, residence, working status, and age: results of analysis of covariance.

ence in the hair mercury level between the mothers with and without artificial hair-waving. Using the multiple regression analysis, the log-transformed hair mercury level was significantly related to the log-transformed daily mercury intake (standardized regression coefficient  $\beta s$ =0.307, p<0.001) and artificial hair-waving ( $\beta s$ =-0.276, p<0.001); but, not to hair coloring/dyeing ( $\beta s$ =-0.065, p=0.21), residence ( $\beta s$ =-0.070, p=0.18), working status ( $\beta s$ =-0.012, p=0.81) or age ( $\beta s$ =-0.046, p=0.37). The hair mercury concentrations adjusted by daily mercury intake and the above factors were significantly higher in the mothers without artificial hair-waving (geometric mean, 1.81 µg/g) than in the mothers with (1.29 µg/g), as shown in Fig. 2; but, no significant difference in the hair mercury was seen between the mothers without hair coloring/dyeing (1.74 µg/g) and with (1.59 µg/g) (p=0.21).

# Discussion

In a previous study, we examined the accuracy of daily

mercury intake estimated from the FFQ data of 154 mothers residing in Akita, Japan (4). Also, another study of the FFQ with 122 food items reported that the correlation coefficients between nutrients estimated by the first and second tests conducted at an interval of one week (i.e., reproducibility) ranged from 0.64 for vegetable protein to 0.78 for calcium (16). In this study using the FFQ, a large number of mothers including those who had different food habits in Japan were investigated, and they were suspected of ingesting mercury amounting to median 15  $\mu$ g/day from seafood and freshwater fish, which was similar to the level of mothers residing in Akita (4). Since the daily mercury intake in our study was significantly correlated with hair mercury levels, daily mercury intake from seafood could reflect the individual methylmercury exposure to some extent.

The principal findings in 327 Japanese mothers of this study were that artificial hair-waving was associated with current total mercury levels in hair, and that permanent hair treatment reduced total mercury in hair by approximately 30% as a mean value, even after adjusting for daily mercury intake, hair coloring/dyeing, and other possible factors (Fig. 2). We used aliquots of hair samples corresponding to the duration of a mean of ten months for the determination of total mercury, whereas we did not have accurate information on when the mothers got their hair permed. In fact, the distributions of hair mercury in the mothers seemed to differ; i.e., open circles indicating a mother with artificial hair-waving, as shown in Fig. 1, were somewhat skewed to the downward direction. Yamamoto and Suzuki (2) explained that thioglycolate in artificial waving lotion removed hair mercury effectively. Likewise, one experiment by Yasutake et al. (3) reported that more than 30% of the hair mercury in four women was removed by a single treatment of the above lotions, and repeated treatments further removed the hair mercury. This error is unmeasurably bigger than analytical imprecisions in the laboratory; the latter has been estimated to be less than 5% (5, 8). For that reason, the myth that mercury concentration in hair reflects the methylmercury concentration circulating in the blood (6, 7) may collapse if a study population includes subjects with such hair treatment.

In the present study, hair coloring/dyeing was not signifi-

cantly associated with current hair mercury levels, and no significant difference in the hair mercury level was observed between the mothers with and without hair coloring/dyeing, although approximately 10% of hair mercury in the mothers with hair coloring/dyeing seemed to be removed when compared with the mothers with natural hair. On the contrary, a preliminary study has reported that the overall average mercury concentration in four subjects was 14.2  $\mu$ g/g for white hair and 15.3  $\mu$ g/g for pigmented hair (5); in another report, total and organic mercury concentrations in Japanese elderly men and women were significantly higher in naturally grey hair than in dark hair (21). Thus, it is likely that when women conceal grey hair by coloring or dyeing, the hair treatment may cause possible differences in the hair mercury level. Further research is necessary to explore whether mercury concentrations in naturally grey hair or in pigmented hair are higher, as well as whether the ratio of mercury concentrations in naturally grey hair and pigmented hair differs with respect to race.

Neither daily mercury intakes nor hair mercury levels in Japanese mothers differed significantly according to residence (Table 2). A similar finding has been observed both between fishing and non-fishing areas and between cities and towns in Akita Prefecture (4). Also, working status was not relevant to the daily mercury intake or hair mercury level. Since no

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mothers residing near the areas where a mine/smelter existed in the past were included, the possibility of environmental mercury vapor binding to the hair was minimal. In this way, the impacts of preanalytical factors except the above hair treatment on hair mercury levels appear to be omitted in the current study.

In environmental epidemiological research, it is essentially impossible to obtain an error-free measurement of exposure. When unavoidable measurement error, i.e. artificial hairwaving, is ignored, it is probable that the estimation of the exposure effects is biased toward the null hypothesis (22, 23). In interpreting epidemiological studies based on hair mercury only, attention should be directed toward the consequences of exposure misclassification. Rather, mercury in blood or methylmercury in cord tissue, together with hair mercury, may be recommended in such studies on risk assessment (9, 12, 24), because the usefulness of the mercury concentration in cord blood has been emphasized as a main risk indicator (25).

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