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Determination of Total Mercury Concentration Level in Hair of the Kuala Lumpur Residents: A Linear Regression Approach

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ABSTRACT

Determination of total mercury level in hair samples of Kuala Lumpur residents was carried out by neutron activation analysis. The average level of total mercury content in hair of the residents was 3.36 mg kg^{-1} in range of 0.59−18.73mgkg^{-1}. A linear regression approach of the influence of factors such as age, fish consumption, race and gender was applied. A positive and significant correlation were found between hair total mercury concentration and fish consumption. A significant difference in total mercury concentration was also found between the three ethnic groups (Malay, Chinese, Indian). In contrast, gender and age were insignificantly contributed to the level of total mercury. However, the interaction between gender, age and fish diet contribute significantly to the level of total mercury in this population.

Kew Words: Neutron activation analysis; Total mercury; Linear regression model; Fish

INTRODUCTION

Mercury has long been known as an environmental pollutant. One of the many questions concerning mercury in the environment is the extent of mercury concentration in humans. Generally, human accumulate mercury species in their bodies via absorption of mercury from their diet (specially, sea food) and from air in their local environment (Airey, 1983). Kuala Lumpur, the capital city of Malaysia, is the most populous, urbanised and industrialised region in the country. Fish and shellfish is the main source of animal protein for the general residents and covers about 80% of the protein requirement of the population. Therefore, the possibility of high mercury level in residents is expected.

Human scalp hair has been widely used as a bioindicator for mercury exposure for human populations (WHO, 1990). It is easy to collect, transport and store (Katz and Chatt, 1988). Its mercury level has been reported to be stable for long time (Suzuki and Yamamoto, 1982). Hair also contains 250 times more mercury than blood (Agency for Toxic Substances and Disease Registry, 1998). Therefore, the level of mercury content in residents of different areas might be obtained by analyses of hair samples.

Fish consumption, country and place of residence, occupation, age, and gender all have some effects on human hair mercury level (Feng et al., 1998; Airey, 1983; Maloney et al., 1998; Dickman et al., 1998; Batista et al., 1996). In this study, hair samples were collect from Kuala Lumpur residents and analysed for total mercury by neutron activation analysis. Linear regression model was used to investigate the influence of the contributory variables such as fish consumption, age, race and gender to the level of total mercury in this population.

MATERIALS AND METHODS

Sample Collection:

Hair samples were collected randomly from 400 hundred individuals of Kuala Lumpur residents. These samples were collected from donors by single cutting from the occipital region with a pair stainless scissors in accordance with the IAEA protocols. During the collection of the hair samples, each individual was asked to complete a questionnaire detailing name, gender, age, occupation and dietary habits.

Hair Preparation and Analysis

After collection of hair samples from donors, the samples were cut to lengths of about 2─5 mm using clean stainless steal scissors. The hair samples were then washed according to the standard procedure which recommended by the IAEA: wash hair in acetone, thrice in water and once more in acetone. The samples were then dried overnight in an oven at 60˚C. About 0.1 g of each prepared hair sample was weighted and sealed in polyethylene envelopes for total mercury analysis by INAA. The standards were prepared by pipetting about 1μl of the standard chemical solution of mercury onto ashless filter paper and packed in polyethylene envelope after careful drying. To prevent mercury losses, about 50 μl of 10% solution of thioacetomide was pipetted into the filter paper. Two samples along with one standard and blank were packed in clean polyethylene envelope for neutron irradiation. Samples, standard and blank were irradiated in the TRIGA reaction of the Malaysian Institute for Nuclear Technology and Research (MINT) for 6 hours in a neutron flux of $2.3.10^{12}$ $n.cm^{-2}.s^{-1}$. The irradiated samples were then cooled for three days prior to counting. After cooling, the samples, standards and blanks were counted for 1800s using HPGe detector. For calculation of mercury concentrations, the 77 kev peak of ^{197}Hg ($t_{1/2}$= 64.1h) and the 279.1 kev peak of ^{203}Hg ($t_{1/2}$=46 day) were used. The gamma ray energy calibration was made with standard sources prior to every set of experiments. The nuclides were quantified by comparing net photopeak area with those of standards. The accuracy of the analytical method was checked by running ten replicates of the certified reference materials IAEA-085 (human hair with an elevated level of mercury) and IAEA-086 (human hair with low level of mercury). The results of the analysis of certified materials are given in Table 1. In comparing our results with the certified values using t-test and F-test, showed no significant differences ($P > 0.05$) between the two means of the results given by the two methods. The precision of the method was tested by ten replicates analysis of the certified reference materials IAEA-085. The precision of the method "expressed in terms of the coefficient of variation for the replicate analysis" was 6.27% for the total mercury.

Table 1. Results of mercury determination in IAEA reference materials ($mgkg^{-1}$)

Standard Reference Materials	This Work	Certified Value	F-test	t-test	P-Value
IAEA-085	25.84 ± 1.62	26.45 ± 1.15[a]	0.504	0.971	0.344
IAEA-086	0.40 ± 0.05	0.38 ± 0.02[a]	0.160	1.174	0.256

[a] Analysed by Horvat and Mandic

Normality Statistical Analysis

The statistical analysis was performed using SPSS and SigmaStat statistical programs. Data was presented as median and 95% and 99% confidence interval. Frequency histograms of total mercury was examined using descriptive statistics. To be sure of using the correct form of statistical evaluation, we based on graphical nature (as starting step) that sheds light on the structure of the data. Figure 1 indicating that the normal distribution of the data is not the case and skewed to the right. Hence, to avoid the erroneous of the statistics interpretation, the median as an average was used to describe the collection data.

EMPIRICAL RESULTS AND DISCUSSION

The empirical results of the survey revealed that 47 percent and 53 percent of the randomly interviewed respondents within the two types of gender have an age of 2-80 years old. This constitute 46 percent, 31 percent and 23 percent of the Malay, Chinese and Indian races, respectively. The mean (± standard deviation) age of the whole population was 32.80 ±15.95 years, and those for ethnic Malays, Chinese and Indians were 33.28 ± 16.31, 33.66 ± 16.26 and 30.61 ±14.68 respectively. Table 2 summarizes the characteristics of the entire population.

Analysis of four hundred hair samples from individuals with no history of occupational exposure to mercury or its compounds were carried out for total mercury contents. The average value of total mercury level in hair samples of the studying population was 3.36 $mgkg^{-1}$. The frequency distribution of the total mercury concentration are shown in Fig 1. The results indicating that 41% were within the range of 0.5-3.0 $mgkg^{-1}$, and 39% were within the range of 3-5.5 $mgkg^{-1}$. Only 20% of the sample remained outside these ranges (Table 3).

Table 2. Population characterizes

Ethnicity	N	%	Age (years)	Male %	Female %
Malay	185	46.25	2 – 80	45.90	54.1
Chinese	125	31.25	4 – 75	49.60	50.40
Indian	90	22.50	3 – 70	53.33	46.67
Total	400	100%	2 – 80	47.25	52.75

Table 3. Frequency distribution for mercury concentration levels

Class	Frequency	%	% Cumulative
0.5 – 3.0	165	41.25	41.25
3.0 – 5.5	156	39.00	80.28
5.5 – 8.0	49	12.25	92.50
8.0 – 10.5	14	3.50	96.00
10.5 – 13.0	8	2.00	98.00
13.0 – 15.5	6	1.50	99.50
15.5 – 18.0	1	0.25	99.75
18.0 – 20.5	1	0.25	100
Total	400	100	

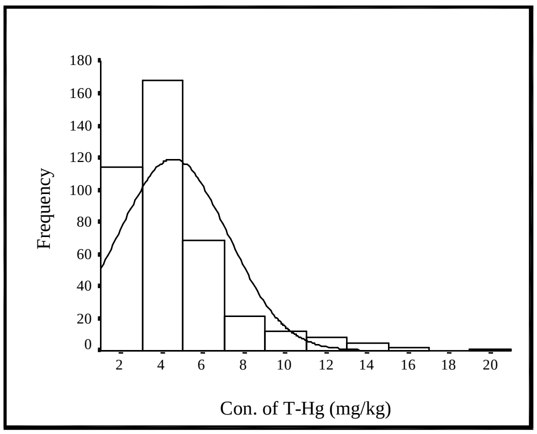

Fig. 1. The frequency distribution of the hair mercury concentrations of Kuala Lumpur population

To estimate the contribution of the race, gender, age, and fish consumption factors to the hair mercury concentration among the individuals of Kuala Lumpur community, a multiple linear regression approach was carried out. An ordinary least squares (OLS) analysis method was used to explain the effects of these factors, which are believed to contribute to the mercury concentration level. In an attempt to estimate the contribution of these factors the following predicted regression model was fitted:

$$Y_i = \beta_0 + \beta_1 D_1 + \beta_2 D_2 + \beta_3 D_3 + \beta_4 X_1 + \beta_5 X_2 + \beta_6 X_1 X_2 + \beta_7 D_3 X_2 + \mu_i$$

Where, Y=Denotes the level of mercury concentration in hair measured in $mgkg^{-1}$; D_1 dummy variable representing Chinese race; D_2 dummy variable representing Indian race. Malay race is control group; D_3 dummy variable for gender (1 = female, 0 = male); X_1=An age of the respondent measured in years; X_2=Refers to the amount of fish diet consumed per respondent per week measured in kg; X_1X_2=Interaction between age and amount of fish diet consumed; D_3X_2=Interaction between gender and amount of fish diet consumed; μ_i =An error term assumed to capture the contribution of other variables, β_0 is a constant, and β_1, β_2, β_3, . . . β_7 are the coefficients of the independent variables that to be estimated; i=Subscript of respondent (i=1,2,…,400).

The results of the correlation of the independent variables with the measured mercury content in hair was considered helpful for finding out to what extent these variables influence the accumulation of mercury in the hair of the subjects. Results of the analysis of multiple regression between the levels of total mercury in hair and the various examined contributing factors are presented in the Table 4. Nevertheless, the results of the diagnostic tests are all satisfactory, those are include the detection of multicollinearity between the independent variables, and the Durbin-Watson test for autocorrelation. In addition we carried out the White test for heteroscedasticity on the equal variance of the error term μ_i; the presence of heteroscedasticity was indicated. As a remedial approach we estimated the results by using heteroscedasticity-consistent standard errors and covariance.

Table 4. Regression results of mercury level in Kuala Lumpur predicted model

Independent variable	Regression coefficient	Standard Error	t-value	P-value	Partial correlation
Constant	-0.09	0.55	-0.16	0.8738	
Chinese race	0.67	0.18	3.70**	0.0002	0.178
Indian race	0.88	0.22	4.06**	0.0001	0.219
Gender	-0.78	0.53	-1.47	0.1428	-0.099
Age	-0.02	0.02	-1.34	0.1794	-0.076
Fish diet	4.97	1.03	4.82**	0.0000	0.297
Age*Fish diet	0.05	0.03	1.79***	0.0736	0.110
Gender*Fish diet	1.94	0.94	2.06*	0.0398	0.166

R^2 = 0.67 Multiple R = 0.82

F-ratio = 115.07** P-value = 0.0000

N = 400; * Significant at 5%; ** Significant at 1%; *** Significant at 10%.

As could be seen from Table 4, the model has a reasonably high explanatory power with coefficient of determination $R^2 = 0.67$. This indicates that 67% of the mercury concentration levels among the individuals of Kuala Lumpur community are explained by the contributing factors under considerations. A high value of multiple R (82%), which measures the correlation between the observed and predicted values of the mercury concentration levels; indicates that there is obviously linear relationship between the mercury concentration levels and the contribution of the causative: race, gender, age and fish consumption factors. Further, the F-value of 115.07 indicates the overall significance of the model at 1%. This means the rejection of null hypothesis that the regression coefficients $\beta_1 = \beta_2 = \beta_3 = ... = \beta_7 = 0$ and the acceptance of the alternative hypothesis that at least one variable coefficient is not equal to zero. Moreover, it indicates that such association between the mercury concentration levels and the contributing factors under considerations could not be of random origin. The estimated regression model is as follow:

$$Y = -0.09 + 0.67D_1 + 0.88D_2 - 0.78D_3 - 0.02X_1 + 4.97X_2 + 0.05X_1X_2 + 1.94D_3X_2.$$

The regression coefficient of a variable measures the change in the mercury concentration levels associated with a unit change in the explanatory variable given that, all other explanatory variables are held constant.

The contribution of Chinese and Indian races to the mercury concentration levels among residents of Kuala Lumpur community were found to be significant and resulted in 0.67 and 0.88, respectively. These high levels of Chinese and Indian races contribution could be attributed to the different culture and life style for each group such as fish consumption frequency and using traditional medicine which contains amount of mercury (Kang-Yum RPh and Oransky MD, 1992; Kew *et al*., 1993).

The gender and age variables were negatively and insignificantly contributed to the concentration levels among residents of Kuala Lumpur community. These findings accord with Gonzalez *et al.* (1985) who found that, as donor's age increase the hair mercury levels appear to decrease but the correlation was not significant. While in other study, no significance difference in mercury content in the hair samples of the gender and age were found (Shrestha & Fornerino 1987). Batista et al. (1996) found a significant correlation between level of mercury in hair and gender ($r=0.27$, $P<0.001$) and in contrast, age did not showing any significant correlation with the hair mercury contribution levels.

According to the World Health Organisation, the average person receive most of their mercury from fish and shellfish diets (WHO, 1991). Being fish constitutes the main source of protein for the great majority of Kuala Lumpur residents, it is important to study the influence of fish consumption on mercury intake by this population. As expected, The amount of fish diet taken per week per resident of this community was highly significant contributed to the mercury concentration levels (4.97). Therefore, variations in total mercury levels in this population are to be attributed to different fish feeding habits. This result confirms that, fish consumption is the main route of total mercury exposure in the Kuala Lumpur residents. This finding confirms and extends previously findings that reported in different parts of the world. Such as: Surmani *et al*., 1994; Oskarsson *et al*., 1994; Abe *et al*., 1995; Leino and Lodenius, 1995; Holsbeek *et al*., 1996; Batista *et al*., 1996; Feng *et al*., 1998; Renzoni *et al*., 1998 and Dickman, 1998.

The interaction terms between gender, age, and the consumed amount of fish diet revealed significant contribution to mercury concentration levels among these residents, 0.05 and 1.93 respectively. In addition, the positive signs of their coefficients indicate that the interacted variables were similarly acted towards their contribution. The females in this population consume more fish than males. Whereas older people generally tended to consume more fish than the younger and as a consequence, higher levels of total mercury in hair samples of the older people were observed.

The estimated results of partial correlation showed that the contribution of the amounts of fish diet intake to the mercury concentration levels in the hair of Kuala Lumpur residents (other variables held constant) ranked first with 30%. This indicating that, fish consumption is the dominant factor, which contributes highly to the level of total mercury in the hair samples of the Kuala Lumpur population. The remains factors according to their contribution ranked as: Indian race (22%), Chinese race (18%), gender and fish diet interaction (17%), and age and fish diet interaction (11%) (Table 4).

formulate appropriate models for the purpose of building explicit, theoretically interesting comparisons into our models. According to the different races and the gender we could construct the models for Kuala Lumpur residents for further studies.

CONCLUSION

The present work shows a marked impact of fish consumption on hair mercury levels in Kuala Lumpur residents confirming that, fish diet is an important source of mercury exposure in this population. Indian and Chinese race also contributed positively and significantly to the overall total mercury level. On the other hand, age and gender negatively and insignificantly contributed to the hair total mercury level. However, significant interaction between age, gender and fish diet also contributed to the total mercury level. According to this study, even among individuals with level of mercury considerably above the PTWI, the hair mercury content in Kuala Lumpur resident does not reach the levels of toxicity. This revealed that, at present, mercury pollution does not appear to be a serious problem in Kuala Lumpur. However, due to the large quantities of fish and other seafood consumed by the Malaysian people in their daily diet, the mercury pollution in Kuala Lumpur and other Malaysian cities should be continuously and crucially observed for head hair mercury level and health.

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