ORIGINAL ARTICLE

Determination of Arsenic and Lead Level in Blood of Adults from Coastal Community in Melaka, Malaysia

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ABSTRACT

Introduction: Accumulation of heavy metals through seafood consumption constitutes a significant potential threat to human health. Biomonitoring of whole blood heavy metals level gave an insight into the internal body burden to the exposure of heavy metals. The aims of this study were to assess the blood heavy metals (arsenic and lead) level among the coastal community of Melaka and to determine their association with sociodemographic background and potential sources of heavy metals accumulation. **Methods:** Respondents were recruited through purposive sampling technique based on inclusion and exclusion criteria. The questionnaires were distributed to obtain sociodemographic information, the frequency of seafood intake and smoking habit. Blood samples were obtained on a voluntary basis. A total of 63 respondents completed all the information required. The heavy metals concentration in blood was determined by inductively coupled plasma- mass spectrometry (ICP-MS). **Results:** The blood arsenic (BAs) concentration of respondents was 0.076 (0.059 – 0.107) µg/L and the blood lead (BPb) concentration of respondents was 1.204 (0.670 – 2.094) µg/L respectively. A significant association was observed between seafood-based product frequency intake and BPb concentration ($p < 0.05$). Other seafood frequency intake and background factors were not significantly associated with the BAs and BPb concentration of respondents. **Conclusion:** The findings showed that arsenic and lead levels in the blood of respondents along the coastal area of Melaka did not exceed the blood heavy metals reference levels and there was also lack of associations between blood heavy metals concentration and the potential factors of heavy metals accumulation studied.

Keywords: Blood Arsenic, Blood Lead, Coastal Community, Whole blood

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INTRODUCTION

Marine pollution by heavy metals is an environmental issue of concern. Anthropogenic activities such as industrialization, municipalities and agriculture increasing intensities are posing a threat to the aquatic environment (1). It was reported that the accumulation of metals in the aquatic environment was due to intensive dredging, reclamation and shipping activities (2).The uncontrolled heavy metal discharged into the sea will negatively affect the natural structures and functions of marine life populations.

Arsenic and lead are among the toxic heavy metals which have been recognized for their negative effects on the environment where they can accumulate throughout the food chain posing serious threat to human health (3). Low level of exposure to heavy metals could cause poisoning and diseases, while high level of exposure could cause immediate serious effects (4). Bioaccumulation of heavy metals in the human body can induce adverse health effects. The chronic exposure to arsenic may cause hyperpigmentation of the skin, heart disease and liver damage (5), while exposure to lead may cause neurotoxicity, hypertension and anaemia (6).

Humans come into contact with different heavy metals primarily via inhalation and ingestion, while dermal contact represents a minor route of exposure (7). Report from literature suggested that seafood consumption was an important route of human exposure to a variety of chemical contaminants (8). Heavy metals, such as mercury, cadmium and lead and polychlorinated biphenyls (PCBs) represent a group of highly toxic substances accumulating in the tissues of marine organisms and were passed down through the food chain to human (9-10).

A study on some locally processed raw seafood products found that every sample contained arsenic with dried shrimp showing the highest value compared to the other samples (11). Low concentration of arsenic was reported in wet weigh samples of some fishes such as the Long-tailed butterfly ray, Gray eel-catfish, Spanish mackerel and prawns along the Straits of Melaka (12). A study in the south-west coast of Malaysia also reported that a few numbers of fishes contained lead in their muscle and gills (13). The bioaccumulation and magnification capability of certain fish and shellfish may result in toxic level even in low concentration (12).

Heavy metals are freely dissolved and readily taken up by fish and other aquatic organisms which eventually accumulate in human body tissue through consumption of the contaminated seafood which may pose serious health effects (1, 14,15). The ingested arsenic and lead through seafood consumption are absorbed through the gastrointestinal tract and enter the bloodstream where they are then distributed to major organs like the kidneys and liver (16-17). Whole blood lead and arsenic concentrations act as the biomarker of exposure in individuals (18). Those with ongoing chronic exposure are best assessed by analysing total blood concentrations. Blood heavy metals level may become a useful biomarker of exposure through seafood intake as it reflects the actual measurements of body burden which will be unaffected by respondents' reporting bias on diet records and recalls (19).

Blood heavy metal levels are also related to sociodemographic factors such as age and gender and lifestyle habit of individual such as alcohol consumption and smoking (20). Arsenic was reported to be in higher concentration in the blood and urine of those living by the coast compared to those living inland (21). The existence of a correlation between seafood consumption and heavy metal excretion has remained controversial. Therefore, it is important to determine the arsenic and lead concentrations in blood samples of the coastal community to monitor and assess their effects on the population. In this study, the levels of elemental arsenic and lead in whole blood samples from respondents were determined and those levels were then correlated with their sociodemographic factors, the frequency of seafood intake and smoking habit of respondents.

MATERIALS AND METHODS

Study Design and Respondents

This cross-sectional study was conducted from September 2014 to February 2016 along the coastal areas of Melaka. The respondents were purposively recruited based on inclusion and exclusion criteria among healthy Malay adult. The inclusion criteria include; living more than one year in the coastal area of Melaka to reflect practices occurring in Melaka coastal community while limiting the effect of other exposures on the biomarkers measured, adult (male/female), age ranging from 18-60 years old and those who consume seafood and seafood based product in their daily diet. Exclusion criteria include residents who were pregnant and just delivered the baby. They were recruited on voluntary basis along the coastal area from three districts of Melaka which included Alor Gajah, Melaka Tengah and Jasin. Respondents living along the coastal area were expected to consume more seafood and seafood product which would mainly expose them to lead (Pb) and arsenic (As).

A total of 63 respondents participated in this study with informed consent and voluntarily participated for further blood heavy metals analysis. The study protocol was approved and conducted in accordance with the Ethics Committee for Research Involving Human Subjects (JKEUPM) as defined by the research institution (Reference number: UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2).

Questionnaires

A set of pre-tested questionnaire was administered by an interviewer (the researcher or the enumerator who has been trained by the researcher). The questionnaire was adapted from the Malaysian Adult Nutrition Survey (22) and was modified following the feasibility of the respondents to gather background information of respondents. The questionnaire consists of three sections which are sociodemographic information, seafood frequency intake which were classified into frequent (if the respondents consume seafood $\geq$ 3 times per week) and less frequent (if the respondents consume < 3 times per week) and other potential sources of exposure to heavy metals which include occupational exposure and lifestyle behaviour such as smoking among respondents. The pre-test was performed before the actual survey takes place. Thirty eight adult respondents were chosen to participate in this pre-test. This pre-test was done to ensure all the questions asked were appropriate and understandable. The questionnaire was tested on respondents with similar characteristics with the study population. The test took place along the coastal area of Klang, Selangor. The questionnaire was modified accordingly before the actual survey being conducted.

Blood Collection and Wet Acid Digestion of Blood sample

Eight mL of whole blood samples were collected in EDTA tubes by medical laboratory technician through the venipuncture procedure and were stored at -20°C before being analyzed within 2 days. The whole blood sample was digested by a wet acid digestion method adapted from Yahya et al. (23). A fresh mixture of 69% nitric acid (HNO3) and 30% hydrogen peroxide (H2O2) was prepared by mixing both reagents in the ratio of

2:1 respectively. An accurate measure of 0.5 ml of whole blood was pipetted into a conical flask. Three ml of HNO_3 and H_2O_2 mixture was added into the blood sample and stood for 10 minutes. The blood mixture was heated on the hot plate at 70°C for 2 hours covered with a glass watch. Two ml of HNO_3 was added into the conical flask while heating continued on the hot plate at 80°C. A few drops of H_2O_2 were added into the conical flask until clear solution obtained. The excess acid mixture was evaporated until a semi-dry mass. The mixture was cooled and diluted with 0.1 ml of HNO_3. Then the mixture was filtered into a 100 ml volumetric flask and filled up till the calibration mark. The procedure was repeated for the preparation of a blank sample.

Determination of Whole Blood Heavy Metals Level

The digested whole blood samples of respondents were analysed for heavy metals level by ICP-MS (ICP-MS 7500c; Agilent Technologies, USA). A standard curve of gradient ± 0.999 (linear through zero) was obtained by using Perkin Elmer's Multi-element Standard Calibration 3. Each sample was triplicated to ensure accurate reading of each sample with minimum error. A minimum sample of 20 mL was used for the analysis.

Statistical Analysis

All the data collected were analysed statistically using SPSS software version 22. Descriptive analysis was conducted to obtain frequency, median, IQR and range of variables. Nonparametric tests were used to analyse the association between the variables due to data not normally distributed in the main continuous data. Spearman Rho correlation test was conducted to find the correlation between the concentration of arsenic and lead in blood with age, household income and duration of settlement. Mann-Whitney U test was employed to find the correlation between the concentration of arsenic and lead in blood with gender, education level, seafood consumption frequency and smoking habit. The value of $p < 0.05$ was considered as statistically significant.

RESULTS

The characteristics of the respondents are presented in Table I. The median age of respondents was 38 (27 – 48) years old ranging from 18 to 60 years old. The median duration of settlement in the study area was 28 (19 - 40) years with the median household monthly income of RM2500 (1500 – 3500). As for gender, a total number of 29 were male and 34 were female. Majority of the respondents (81%) had secondary and lower education level which include those who finished their Ujian Penilaian Sekolah Rendah (UPSR) only and those who managed to finish their study level up to Penilaian Menengah Rendah (PMR) and/or Sijil Pelajaran Malaysia (SPM). On the other hand, 19% of the respondents have tertiary and higher education level which include those who managed to finish their certificate, diploma, undergraduate, and postgraduate studies. Majority of the respondents were a frequent eater of fish (92.1%) and concerning smoking habits, 34.9 % of respondents were smokers.

Table I: Characteristics of total respondents (n=63)

Variables	Median (IQR)	N (%)
Age (Years)	38 (27- 48)	
Duration of Settlement (Years)	28 (19 - 40)	
Household Income (RM)	2500 (1500 – 3500)	
Gender:		
Male		29 (46.0)
Female		34 (54.0)
Education Level:		
Secondary and lower		51(81.0)
Tertiary and Higher		12(19.0)

Heavy metals levels in the whole blood sample of respondents are presented in Table II. The concentration of BAs of the respondents in this study was 0.076 (0.059 – 0.107) µg/L within the range of 0.01 to 0.36 µg/L. The median concentration of BPb in respondents was 1.204 (0.670 – 2.094) µg/L within the range of 0.06 to 5.06 µg/L.

Table II: Heavy metals concentration in blood sample (µg/L)

	Arsenic	Lead
Median (IQR)	0.076 (0.059 – 0.107)	1.204 (0.670 – 2.094)
Range	0.01 - 0.36	0.06 - 5.06
Reference Value	10[a]	50[b]
Statistical Analysis*	$t(62) = -1475.16$, $p< 0.05$	$t(62) = -346.10$, $p< 0.05$

[a] reference value of arsenic in blood by ATSDR (24)
[b] reference value of lead in adult blood by CDC (25)
* One sample t-test

Factors associated with the increase levels of blood arsenic and lead were shown in Table III. The BAs concentration was slightly higher among females 0.081(0.067 – 0.109) µg/L than males 0.069 (0.056 – 0.098) µg/L while the BPb concentration was higher among the males 1.656 (0.744 – 2.441) µg/L than the females 1.130 (0.560 – 1.893) µg/L. The blood heavy metals concentrations were higher among those with tertiary and higher education level compared to those with secondary and lower education level. As for the seafood intake, it was found that BAs concentration was higher among those who consumed squid and prawn frequently while the BPb concentration was higher among those who ate less frequently seafood-based product such as fish cracker, prawn cracker and shrimp paste. It was also found that both BAs and BPb concentrations were higher among the respondents who smoke.

The associations of heavy metals concentration in blood with sociodemographic factors, seafood intake and smoking habit were shown in Table IV. There was a significant association between BPb level with seafood-based products which showed p-value < 0 .05. There were no significant associations between BAs and BPb level with other tested variables.

Table III: Factors associated with increased levels of blood arsenic and lead concentration (n= 63)

Factors	Frequency (N)	Median (IQR)	
		BAs (µg/L)	**BPb** (µg/L)
Gender			
Male	29	0.069 (0.056 – 0.098)	1.656 (0.744 – 2.441)
Female	34	0.081 (0.067 – 0.109)	1.130 (0.560 – 1.893)
Education Level			
Secondary and lower	51	0.074 (0.056 – 0.107)	1.075 (0.670 – 2.320)
Tertiary and higher	12	0.087 (0.069 – 0.105)	1.623 (0.758 – 2.010)
Fish			
Frequent	58	0.077 (0.059 – 0.108)	1.300 (0.691 – 2.091)
Less frequent	5	0.067 (0.061 – 0.091)	0.838 (0.396 - 2.403)
Squid			
Frequent	15	0.080 (0.056 – 0.124)	1.616 (0.698 - 2.652)
Less frequent	48	0.076 (0.059 – 0.107)	1.175 (0.654 - 2.087)
Prawn			
Frequent	15	0.080 (0.056 – 0.124)	1.616 (0.698 - 2.652)
Less frequent	48	0.076 (0.059 – 0.107)	1.175 (0.654 - 2.087)
Seafood based Products			
Frequent	18	0.075 (0.057 – 0.107)	0.660 (0.442 - 1.567)
Less frequent	45	0.076 (0.061 – 0.107)	1.660 (0.806 - 2.315)
Smoking			
Yes	22	0.072 (0.056 – 0.110)	1.695 (0.702 - 2.698)
No	41	0.077 (0.064 – 0.107)	1.113 (0.611 - 1.820)

Table IV: Association of heavy metals concentration in blood with sociodemographic characteristics, frequency of seafood intake and smoking habit

Factors	BAs		BPb	
	Statistical Analysis	p-value	Statistical Analysis	p-value
Sociodemographic: Age[a]	r = - 0.033	0.796	r = - 0.174	0.172
Household income[a]	r = 0.141	0.270	r = - 0.038	0.769
Duration of settlement[a]	r = - 0.131	0.304	r = - 0.047	0.713
Gender [b] Male Female	U = 373.0	0.098	U = 415.0	0.282
Education Level [b] Secondary and lower Tertiary and higher	U = 229.5	0.180	U = 294.0	0.834
Seafood Intake[b] Fish Frequent Less frequent	U = 119.5	0.517	U = 121.0	0.542
Squid Frequent Less frequent	U = 341.0	0.759	U = 330.0	0.628
Prawn Frequent Less frequent	U = 341.0	0.759	U = 330.0	0.628
Seafood based products Frequent Less frequent	U = 383.5	0.744	U = 233.0	0.009*
Smoking[b] Yes No	U = 389.5	0.375	U = 370.0	0.243

* Significant at $p < 0.05$ [a] Spearman rho test [b] Mann Whitney U test

DISCUSSION

There were significant differences in BAs and BPb concentrations of the respondents and the reference value of heavy metals in blood; BAs is less than 10 µg/L (24) and BPb is less than 50 µg/L (25) where p<0.05 respectively. The concentration was consistent with a study in Ghana which reported a low concentration of BAs (0.032 ± 1.12 µg/L) among respondents who lived in a non-mining area (26). The BAs level was much lower compared to the study in other countries where Norway reported BAs level of 5.9 µg/L (IQR: 40.2 µg/L) among the respondents with high consumption of fish (21), Southern Taiwan reported BAs level of 7.41 ± 4.70 µg/L among respondents who avoided eating seafood before the blood collection (27) and Finland which reported BAs level of 0.47 ± 2.78 µg/L (20). The median concentration of BPb in respondents was 1.204 (0.670 – 2.094) µg/L and is within the range of 0.06 to 5.06 µg/L which was similar to a study in Ghana which reported BPb concentration of 2.40 µg/L (26). This low concentration was in contrary to other studies among the Taiwanese, Finnish and Korean adults which reported BPb level of 24.46 ± 9.69 µg/L (27), 12.44 ± 2.16 µg/L (20) and 22.2 µg/L (28) respectively. The differences in BAs and BPb levels among countries may be due to other factors such as lifestyles, dietary habits and different inter individual metabolism and variation in eliminating foreign intermediates. The efficient metabolism and excretion capability among respondents in Melaka might be the reason for their low level of heavy metals detection in blood.

Present study showed no significant correlations between BAs level and age which was consistent with a study in Brazil among As-exposed residents (29). There was also no significant correlations between BPb level and age among respondents which is in contrary to the studies in Spain, Australia and France which reported BPb level increased linearly with age (30-32). These studies demonstrated the cumulative nature of lead exposure over years among respondents. There was no significant correlation between BAs level and household income which was in contrary to (29) that reported higher level of As is associated with lower household income. The BPb level of respondents was found not correlated to household income. This finding was in contrary to other study which observed a negative association between BPb level and socioeconomic status of respondents (32).

There was no significant correlation between the BAs level and gender which was an agreement of those observed by Khlifi et al. (33). However, a trend can be seen in which females had a higher median BAs level compared to males. Present study is similar to Falq et al. where males were observed to have higher BPb level compared to females due to their lifestyles which involved smoking and alcohol consumption and also occupational exposure to heavy metals (32). The educational level had no significant correlation to BAs level in this study. This is similar to a study by Ugwuja et al. (34) which reported that there was no significant relationship between educational level and plasma As levels in respondents. The BPb level was also found not to be significantly correlated to educational level which is in contrary to Abass et al. which reported BPb levels were elevated among those with lower educational level (20).

It was observed that there were no associations between BAs levels and the frequency of seafood and seafood-based products intake. This study's finding was contrary with studies in Brazil, Finland and Norway where high As concentration was related to high consumption of seafood among the respondents (21, 20, 29). Although 90.5% of respondents in this study had frequent seafood intake, the level of BAs still did not exceed the guideline levels. This might be due to the low level of As from the seafood sources in the coastal area of Melaka. There was a significant correlation between frequency of seafood-based product intake and BPb level where less frequent eater of the seafood-based product had higher BPb level, suggesting contribution of other sources of exposure to Pb aside from seafood-based product.

Smoking habit among the respondents was also found not significantly correlated with BAs concentration which is similar to a study by Freire et al (29). This is contrary to another study where those who smoked had a higher BAs concentration than those who did not smoke (35). Smoking habit of respondents also showed no significant correlation with BPb level. This was in disagreement with other studies which reported smoking was related to Pb contamination (20, 32, 36). This could be due to the different smoking behaviour between the respondents (37) where most of the respondents in Melaka were reported as light smokers and smoked less than 10 cigarettes in a day.

Further assessment on heavy metals in seafood taken by respondents which include As and Pb analysis of the most frequently eaten fish by the respondents would be essential to confirm the observations reported. The low correlations could be due to the low accuracy of the data contained in the questionnaire, where present study provided only approximate information on seafood frequency intake and not a detailed measure of the seafood consumed by respondents. The correlations could be low due to variation of As and Pb levels in portions of different types of seafood ingested. A 24-hour dietary recall may also be useful in assessing the specific recent intake of seafood and seafood-based products of respondents in addition to only determining the frequency of seafood intake. The limited sample size due to the invasive sampling procedure did not permit us to precisely determine whether any correlations exist between blood heavy metals concentration and factors associated with an increased level of heavy metals. A comprehensive study which involves the assessment of urine, hair and nails as a more appropriate biological marker of long-term heavy metals exposures should be considered in addition to blood sampling. Identification of the polymorphism in the enzyme responsible for heavy metals excretion is also important in determining its relation to heavy metals concentration in blood.

CONCLUSION

The concentrations of blood arsenic and lead in this study have not exceeded the reference value levels which indicated that heavy metals exposure, in the range of levels observed along the coastal area of Melaka did not pose a major threat to the respondents' health. The present study found that there were lack of significant associations between blood heavy metals (arsenic and lead) concentration with the sociodemographic background, the frequency of seafood intake and smoking habit of respondents. The monitoring of heavy metals concentrations in the community is important to assess their impact on human health.

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