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Methylmercury Detection in Maternal Blood Samples by Liquid Chromatography with Inductively Coupled Plasma Mass Spectrometry

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ABSTRACT

Methylmercury (MeHg) is one type of mercury (Hg) species known to be very toxic to humans, especially pregnant women and their fetuses. This study aims to obtain and validate the optimum condition of liquid chromatography with inductively coupled plasma mass spectrometry (LC-ICP-MS) to test MeHg concentration. To date, there is limited research that is focused on the maternal blood MeHg samples using LC-ICP-MS in Malaysia. Before analysis, collected blood (500 µL) was placed into a 15 mL polypropylene test tube, followed by the addition of extractant solution [0.10% (v/v) HCl + 0.05% (m/v) L-cysteine + 0.10% (v/v) 2-mercaptoethanol] to the sample and sonicated for 15 minutes. The MeHg level was detected from the sample solution using the LC with Zorbax Eclipse XDB-C18 (4.6 x 12.5 mm, 5 µm) (Agilent Technologies) guard column and analytical column (4.6 x 150 mm, 5 µm) and was quantified by using the ICP-MS. The recovery of MeHg was in the range of 106 to 112% with RSD of less than 10%, followed by the LOD and LOQ

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values of 0.216 and 0.766 µg/L, respectively. The MeHg appeared at a retention time of fewer than 5 minutes. The results reported that the median (IQR) of maternal blood MeHg level in Malaysian pregnant women was 1.70 (8.90) µg/L, which is 9.7% lower than the LOD value and 11.2% higher than the guideline value of 3.5 µg/L of MeHg in maternal blood.

Keywords: LC-ICPMS, maternal blood, methylmercury determination, validation

INTRODUCTION

Mercury (Hg) is a heavy metal that exists ubiquitously in the environment. It has been listed as one of the heavy metals with evidence and proven to cause detrimental health effects to humans. The Hg toxicity to its target organs in human varies according to species and forms, resulting in a different range of toxicity, including carcinogenicity, mutagenicity, and teratogenicity (Jeevanaraj et al., 2015). Organic Hg compounds are more toxic than inorganic Hg compounds due to the lipophilic characteristics and chemical properties of organic Hg that makes it penetrate the membrane and enter the cells easily (Baharuddin et al., 2012). MeHg is one of the organic forms of Hg species known to be very toxic to both humans and the environment. MeHg is derived from the methylation process of Hg through microbes' reaction, especially in the aquatic ecosystem (Jeevanaraj et al., 2016).

Exposure to Hg and MeHg poses a threat to human health, especially among susceptible individuals such as pregnant women. MeHg is a toxic organic compound that targets the brain and can across the blood-brain and placental barriers, thus are especially detrimental to the fetuses during the developmental stage (National Research Council, 2000b). Based on the previous studies that conducted the Hg and MeHg exposure in maternal blood and cord blood, the results indicated that the concentration level was higher in cord blood compared to the maternal blood (Soon et al., 2014; Huang et al., 2017; Lee et al., 2010). This observation is due to the high affinity of Hg and MeHg that crosses the placenta. The phenomenon is also expected in the present study; however, we found there was difficulty in sampling the cord blood sample due to the different location of respondent's give birth which is outside of Selangor state.

Several areas in Malaysia, such as West Port, Straits of Malacca, Prai, and Johor, were reported to have a high concentration of Hg in the environment due to the anthropogenic metal loads from industrial activities (Praveena et al., 2013). Hg levels in humans is affected by the dietary fish intake, environmental conditions, and different geographical areas. The West Coast of Peninsular Malaysia showed high concentration of Hg levels contaminated in food (Praveena et al., 2013). In a specific case study, Abdullah et al. (2015) conducted a study of Hg and MeHg accumulation in several fish species in the Manjung coastal area. This area can be influenced by the rapid development from anthropogenic and agricultural

activities, waste and toxic effluent, quarries, and residential developments. The authors found that the levels of Hg and MeHg in fish caught were ranged from 65.13 to 106.10 μg/kg. Although the levels were below the guideline limit set in the Malaysian Food Act (1983) (1000 μg/kg), the higher consumption of fish and marine products could affect the Hg accumulation in the body. Therefore, fish consumption from this area is a major concern to pregnant woman in Malaysia due to great pollution from anthropogenic activities.

The previous studies had attempted to analyze the presence of MeHg in multiple media such as sediment, water, and other biological samples. However, there is limited research focusing on the maternal blood MeHg samples using LC-ICP-MS in Malaysia. The detection of MeHg concentration in biological samples such as blood, hair, nail, and urine were determined to assess the degree of MeHg exposure and determine the detrimental health effects that could have happened. However, the Hg concentrations in both blood and hair are accepted as the valid biological samples of MeHg exposure (Mergler et al., 2007), although both are providing a different reflection of exposure (National Research Council, 2000b). In this study, the detection of MeHg concentration in the blood sample was conducted to assess the degree of exposure in the maternal blood among pregnant women. The concentration in maternal blood will reflect the MeHg exposure in the fetuses. According to Kim et al. (2011), the MeHg concentration in the fetuses was higher than in mothers. Based on maternal exposure, the researchers are able to estimate exposure concentration in the fetus and calculate the health risk assessment of Hg exposure.

The problem statement of this study was there is no data available for methylmercury exposure among pregnant women in Malaysia. Table 1 shows the distribution of MeHg concentration among pregnant women population found in other studies. Besides that, the investigation on Hg exposure level from fish consumption found in the hair that was conducted in Petaling District among women at reproductive age showed that 40.9% of them had exceeded the recommended dose of 0.1 μg/g (Jeevanaraj et al., 2015). This finding propels the current study to focus on the Petaling District to examine the MeHg concentration level from fish consumption. A recent Malaysian study on the Hg exposure from marine fishes which obtained from the wholesale market of the Fisheries Development Authority of Malaysia (LKIM) and fisherman's market in Selangor, showed that seven marine fish species such as Spanish mackerel, golden snapper, torpedo scad, four-finger threadfin, pale-edged stingray, sin croaker and red snapper had total Hg concentration exceeding the FAO/WHO recommendation value of 0.5 mg/kg, with the maximum concentration of 0.90 mg/kg was reported in golden snapper (Jeevanaraj et al., 2016).

The standard methods used to detect the MeHg or Hg concentrations are by using gas chromatography (GC) or by using the high-performance liquid chromatography (HPLC) together with an elemental specific detector such as inductively coupled plasma mass spectrometry (ICP-MS) (Rodrigues et al., 2010). However, the liquid chromatography

(LC) was the most selected separation technique used for Hg speciation compared to the GC because there is no necessity to derive Hg species into volatile compounds before the separation process (Rodrigues et al., 2010). This paper aimed to validate a simple extraction method using the LC-ICPMS technique to detect MeHg in the maternal blood sample, as modified from Rodrigues et al. (2010). The validation step represents a tool essential to prove the claimed function or a specific analytical method to measure the samples for the desired purpose (Tanase et al., 2006) and ensure reliable analytical data.

Table 1

Distribution of MeHg concentration among worldwide population found in other studies

Location	Population	Median	Mean	Range	References
Selangor, Malaysia	Pregnant women – maternal blood	1.70	1.98	0.11 – 9.90	Present study
Charleston, South Carolina, USA	Pregnant women – early gestation	-	0.58	0.01 – 2.70	Donohue et al. (2018)
	Pregnant women – late gestation	-	0.46	0.01 – 2.10	
Seoul and Busan, Korea	Pregnant women – maternal blood	-	2.60	NA	Kim et al. (2011)
St. Lawrence, Canada	Pregnant women – first trimester	-	0.36	NA	Morrissette et al. (2004)
	Pregnant women – second trimester	-	0.30	NA	
	During give birth	-	0.23	NA	
Sweden	Pregnant women – early gestation		0.94	< LOD – 6.8	Vahter et al. (2000)
	Pregnant women – late gestation		0.73	< LOD –2.8	
Korea	Pregnant women	4.05		3.81 – 4.32	Wells et al. (2016)
Mexico	Pregnant women – all trimesters		3.40	Min=0.29-0.43 Max=11.89-31.15	Basu et al. (2014)

MATERIALS AND METHODS

Study Location and Subjects

The study location was selected based on the data from the previous study by Jeevanaraj et al. (2016) that showed 40.9% of women at reproductive age in the Petaling District had MeHg concentration exceeding the recommended EPA RfD (1 μg/g of hair THg) in their hair samples. Thus, the present study has selected study subject based on the following criteria: pregnant women aged from 20 - 49 years old, with the stage of pregnancy from 12 - 40 weeks and from the 7 Maternal and Child Health Clinics based in the Petaling District. The sample size estimation for one group was used to calculate the sample size and the formula was adopted from Lemeshow et al. (1990). After substituting the values into the formula, the desired sample size of this study was 211 after considered the 20% non-response rate. The area probability sampling technique was used to recruit the respondents from each clinic. The clinics were divided into three groups: low, medium and high density, classified based on the density of the respondents who visited the clinic. After classified the area, the sample size for each clinic was calculated until it reached the desired sample size of 211. The sample collected from each area was presented in the Table 2.

Table 2

Selection of Respondents with Probability Proportionate to Size Method

Clinic (Sub-District)	Estimated number of pregnant women in the clinic	Cumulative number of pregnant women in the clinic	Cluster number	Total number of respondents from each clinic
Low density				
Batu 13, Puchong	1,123	1541	1	10
Total	1,541			
% Representative	1,541 / 27, 092 = 5%		1/21 = 5%	
Medium density				
Sri Kembangan	3,372	6,979	2 – 5	40
Taman Medan	3,690	10,669	6 – 8	30
Kelana Jaya	3,732	14,401	9 & 10	20
Batu 14, Puchong	3,826	18,227	11 – 13	30
Total	14, 620			
% Representative	14,620 / 27, 092 = 54%		12/21 = 56%	
High density				
Seksyen 19	4,277	2,2504	14 – 17	40
Seksyen 7	4,588	2,7092	18 – 21	40
Total	**8,865**			
% Representative	8,865 / 27, 092 = 33%		8/21 = 38%	210 ≈211

Chemicals and Reagents

All the plastic and glassware materials used in this study were soaked in 10% (v/v) nitric acid (HNO_3) for 24 hours before rinsing five times with ultra-pure water and then dried using nitrogen gas. The purpose of soaking the plastic and glassware in acid is to remove residues entirely from glassware that may contaminate the sample during analysis. A 1000 mg/L standard solution of Methylmercury Chloride (CH_3ClHg) in H_20 was obtained from Alfa Aesar. The calibration standard was freshly prepared on daily basis over the range of 0.0-20.0 µg/L through serial dilution of the stock solution. The other chemicals for the detection were the trace metal grade hydrochloric acid (HCl) (34-37% v/v) Thermo Fisher Scientific, USA), while L-cysteine mixture (97% m/v), 2-mercaptoethanol (≥ 99.9% v/v), ammonium acetate (99.99% m/v) and HPLC grade methanol (≥ 99.9% v/v) were obtained from Sigma-Aldrich (USA).

Standard Reference Material

NIST SRM 955c Toxic Metals in Caprine Blood was purchased from the National Institute of Standard and Technology (NIST, USA) that includes the mass fraction certification of the total organic and inorganic Hg contents in the blood sample.

Instrumentation

An Agilent LC 1260 was interfaced to the Agilent ICP-MS 7900 series, consisting of the essential compartments for the analysis, the isocratic pump, the automatic sampler, the degasser, and the column compartment. The MeHg species were separated using the LC with Zorbax Eclipse XDB-C18 (4.6 x 12.5 mm, 5 µm) (Agilent Technologies) guard column and analytical column (4.6 x 150 mm, 5 µm) followed by the quantification using the ICP-MS. The signal identification was achieved by comparing the retention time (RT) of the standards and analytes, while the matrix spike (i.e., blood sample with known concentration) was used to confirm the peak standard and analytes in the samples. The MeHg level was quantified using a single ion at m/z 202 for Hg against the calibrated external standards.

The instrument was tuned using 1 µg/L tuning solution from the ICP-MS Stock Tuning Solution diluted with ultra-pure water. The instrument was optimized daily, and the performance checking was conducted for optimum performance of the instrument. For better results, the LC Column (Zorbax Eclipse XDB-C18) was preconditioned by pumping the methanol (HPLC grade) at 0.4 mL/min for at least 2 hours. Next, the column was conditioned with the mobile phase solution using the same flow rate and lasted for at least 30 minutes. The operational conditions of the instrument were shown in Table 3 and 4.

Table 3

Operational Condition (Agilent LC 1260 Isocratic Pump)

Setting Items	Setting Values
Column	Zorbax Eclipse XDB C18, 4.6 x 150 mm, 5µm
Guard column	Eclipse XDB C18 4.6 x 12.5 mm, 5µm
Column temperature	Ambient
Mobile phase	0.05% (v/v)2-mercaptoethanol, 0.4% (m/v) L-cysteine, 0.06 mol/L ammonium acetate and 5% (v/v) methanol, (pH 6.6-6.7)
Mobile phase Flow rate	1 ml/min
Injection volume	100 µL
Run time	10 min (600 sec)
Measurement	Peak high

Table 4

Operational Condition (Agilent Technologist ICP-MS 7900)

Setting Items	Setting Values
RF power	1200 W
Dilution gas	0.40 – 0.60 L/min
Carrier gas	0.6 - 0.8 L/min
Sampling depth	10.0 – 11.0 mm
Nebuliser	Miramist
Nebuliser gas flow	0.5 rps
Spray chamber	Quarts Scott style spray chamber (2ºC)
Interface cones	Platinum sampler and skimmer cones
Isotopes	^{202}Hg
Gas mode	He mode

Extraction Method

The sample extraction was carried out by referring to the previous methods described by Rodrigues et al. (2010) with slight modification. A total of 500 µL of NIST SRM 955c toxic metals in caprine blood (Level 3) was placed into a 15 mL polypropylene test tube. The extraction solution (0.05% of L-cysteine, 0.10% of 2-mercaptoethanol, 0.10% of HCl) was added to the polypropylene tube until the markup volume of 5 mL and then sonicated for 15 minutes. The sample was then centrifuged for 5 minutes at 3500 rpm and filtered

through a 0.22 µm Nylon filter. Finally, the supernatant was placed in the vial for the LC-ICPMS analysis.

Preparation of Calibration Standard

The working standard of MeHg was freshly prepared by diluting the methylmercury chloride 1000 mg/L (Alfa Aesar) in water solution (i.e., stock solution in the extractor solution). A linear equation was obtained by plotting the peak area against the standard concentration at seven points ranged from 0 to 20 µg/L. A calibration curve was prepared using the Agilent Mass Hunter software by plotting the peak high of the standards versus the concentration. The calibration curve was shown in Figure 1.

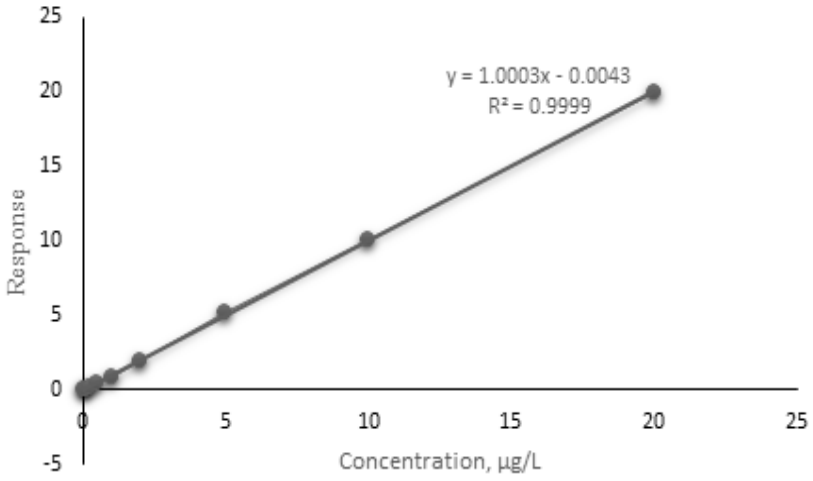

Figure 1. Calibration curve of MeHg Chloride (Standard) (0-20 µg/L)

Method Validation

The method was validated by using the NIST SRM 955c toxic metals in caprine blood, as shown in Table 5. The value observed in the current method was in good agreement with the established target value for the SRM 955c. The LOD was calculated as the mean blank plus with three times the standard deviation of blank, while the LOQ was calculated as the mean blank plus with ten times the standard deviation of the blank (Association of Analytical Committees, 2002). For results below the LOD, the value was replaced by divided by square root of two (Shim et al., 2017; Patel et al., 2019; Taylor et al., 2016; Jo et al., 2015; Gil et al., 2011).

Table 5
MeHg in the NIST SRM 955c. The values obtained are indicated as mean (SD), n=10

Sample	Target Value	LC-ICP-MS method
	MeHg concentration (µg/L)	MeHg concentration (µg/L)
NIST SRM 955c	4.5 ± 1.0	4.6 ± 0.4

Application of Method

A total of 215 blood samples were collected from pregnant women who visited seven Maternal and Child's Health Clinic from the Petaling District of Selangor under the Ministry of Health Malaysia. Three mL of the blood sample was collected from each respondent by the nurse in the clinic following the best practice of the blood withdrawn procedure. The sample was transferred into the K_2EDTA vacutainer blood tube with a lavender cap containing anticoagulant and then inverted several times to mix with the anticoagulant to avoid the blood clot. The blood samples collected from the respondents were immediately placed in a cool box equipped with coolant to maintain the low temperature and transported to the Environmental Health Laboratory of UPM. The samples were stored at -20°C in a deep freezer until further analysis. The samples were extracted and quantified using the same method as described for NIST SRM 955c.

Quality Control

All 20 samples were analysed under the same analytical condition and assigned as one batch for quality checking. All parts of the equipment in contact with samples and reagents were demonstrated to ensure the interference-free before conducted the analysis.

Statistical Analysis

The SPSS statistical software Version 23 was used in statistical analysis. The median value was used in the analysis due to the non-normality of the data.

Ethical Consideration

The study was ethically approved by the UPM's Ethics Committee for Research Involving Human Subject (JKEUPM) (Ref: UPM/TNCPI/RMC/1.4.18.2) and by the Medical Research and Ethics Committee (MREC) with registered National Medical Research Registration (NMRR) ID of 16-782-30590.

RESULTS

This method validation was performed by using the LC-ICP-MS to detect MeHg in the blood samples. To the best of our knowledge, this was the first study reported in the Selangor state of Malaysia to determine the MeHg compound in blood samples. The validation was performed to ensure and confirm that the criteria used were in an acceptable range for the intended use of the MeHg detection in the blood sample using the LC-ICP-MS. The R^2 value of more than 0.995 indicates a good and stringent linear relationship between the concentration and the corresponding peak area.

The performance of an instrument was evaluated by calculating the LOD and LOQ values. The LOD value is determined at the point where the minimum analyte concentration can be detected and reliably distinguished from zero using the instrument. The LOD value was determined by multiplying the standard deviation by 3. The LOQ value refers to the point with the lowest analyte concentration determined quantitatively with an acceptable precision level and accuracy. The LOQ value was measured as ten times the standard deviation from LOD (Shrivastava & Gupta, 2011). The analytical method validation was shown in Table 6.

The acceptable range of sample recovery is from 70 to 120%, with an RSD value of ≤ 20 (Wadhwa et al., 2015; Olmedo et al., 2010). The recovery range in this study was 106 to 112% with the RSD value of < 10% and shows that the method can perform maximum extraction repeatedly, and the validated method is applicable for blood sample extraction using the LC-ICP-MS. Figure 2a and 2b show the chromatograms of MeHg species in the standard solution and blood sample.

The method validation was used to detect the maternal MeHg in blood samples collected among pregnant women from one of the districts in Selangor, Malaysia. The results are given in Table 7. The median (IQR) value was 1.70 (8.90) µg/L, and the geometric mean (GM) value was 1.98 µg/L. A total of 11.2% of pregnant women have exceeded the recommended RfD of 3.5 µg/L MeHg in the maternal blood sample. The range showed that the lowest detected value was one-fold lower than that of the LOD value. The data also showed that nearly 10% of the respondents had a lower detection value than the calculated LOD value.

DISCUSSION

The rapid growth of development and human activities in Malaysia has contributed to contamination and human exposure. The method validation was used to detect the MeHg in maternal blood sample among pregnant women in Selangor, Malaysia. Previously, Jeevanaraj et al. (2016) have found that almost 50% of women in their reproductive age in the Petaling district had exceeded the limit of Hg in their hair which indicated an alarming level of Hg exposure through fish consumption. This current study was conducted among

Table 6

Analytical method validation estimation

	MeHg analysis
LOD (µg/l)	0.216
LOQ (µg/l)	0.766
Calibration range	0.0 – 20.0 µg/L
Linear equation	y = 1.003x – 0.0043
R2 ± SD	0.999 ± 0.001
Precision (% RSD) - Repeatable - Reproducible	 0.332 4.799
Recovery (%)	112 ± 4.23

Table 7

Maternal blood MeHg concentration in respondent (N=215)

	MeHg levels (µg/L)
Range (µg/L)	0.11 – 9.90
Total below LOD, n (%)	21 (9.7)
Average value (µg/L)	1.98
Median (IQR) (µg/L)	1.70 (8.90)
Total Exceeded RfD, n (%)	24 (11.2)

Note. IQR = Interquartile range, RfD = Reference dose

pregnant women, the susceptible group vulnerable to sensitive compound/chemicals such as Hg and MeHg. Selangor state was chosen as the location of the study because it is located in the Klang Valley area, which is known to contribute to environmental issues such as pollution and lead to health problems (Suhaimi et al., 2020; Shahrir et al., 2019). The Petaling district was chosen based on the previous study conducted by Jeevanaraj et al. (2016), who reported the findings of Hg exposure via fish consumption among women in the Petaling area. This study was conducted to investigate the MeHg exposure in pregnant women, one of the Hg species which is known to be very toxic to human health.

Our study found that 11.2% of pregnant women in this study had maternal blood MeHg above the guideline limit of 3.5 µg/L. The United States of Environmental Protection Agency (USEPA) has revised the RfD for Hg in cord blood from the reference dose of 58 µg/L, as recommended by the National Research Council (2000a). After considering the effects of in utero MeHg exposure to child development from the Faroese, New Zealand

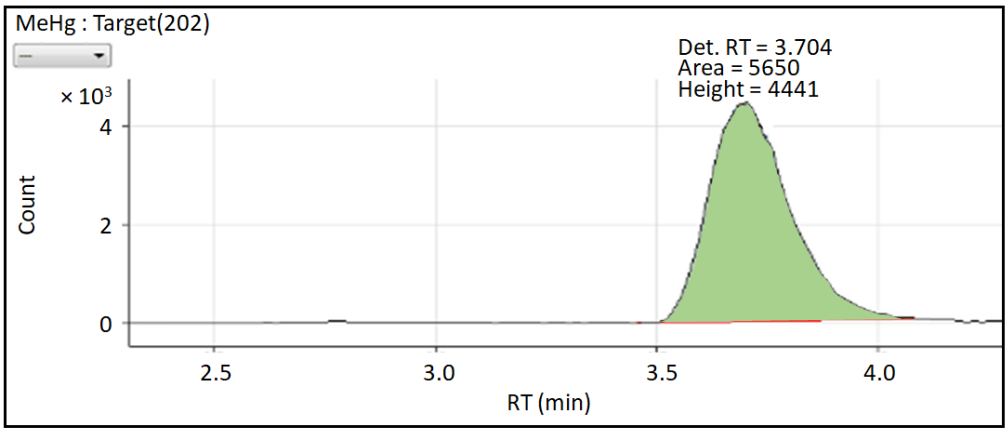

Figure 2a. A standard solution that contains MeHg under an optimised condition with a retention time of MeHg at minute 3.50

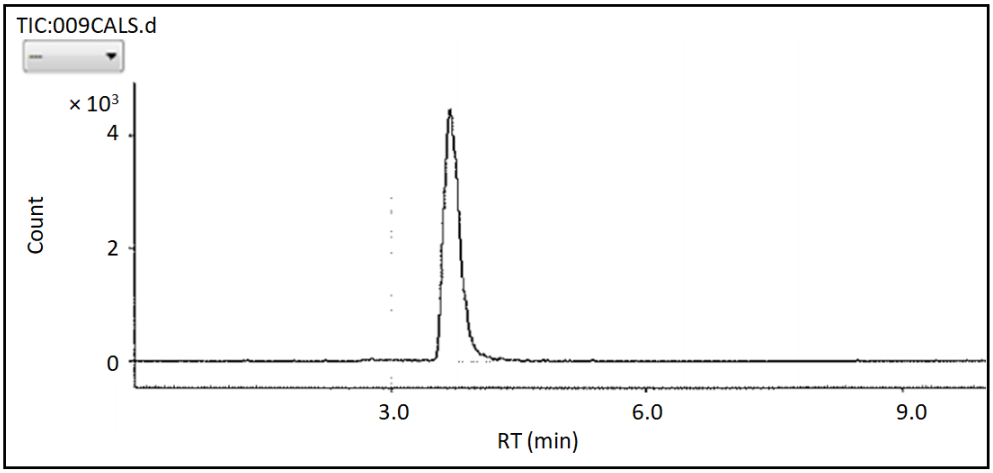

Figure 2b. Chromatogram showing the peak of MeHg species in the blood sample

and Seychelles cohort studies, USEPA has adopted the use of 10 uncertainty factor (UF) value to calculate the 5.8 µg/L Hg RfD in cord blood (Rice et al., 2003). The RfD of 5.8 µg/L Hg in cord blood indicates the association with increased risk of learning disabilities in fetuses. However, Stern and Smith (2003) suggested that the maternal blood Hg level should be revised to 3.5 µg/L, as cord blood levels are on average 70% higher than maternal blood levels (Mahaffey et al., 2004). Previous studies by Basu et al. (2014), Razzaghi

et al. (2014), Donohue et al. (2018), Miranda et al. (2011), Mortazavi et al. (2017), and Silbernagell et al. (2011) used this guideline limit to associate their maternal blood Hg and MeHg exposure levels. Meanwhile, Cusack et al. (2017) has suggested that this guideline limit might be the most applicable guideline for comparison until an updated guideline limit for maternal blood MeHg concentration is determined.

The concentration of maternal blood MeHg in this study was compared to other population studies conducted worldwide. The blood MeHg concentration reported in this study was higher when compared to the studies by Donohue et al. (2018), Morrissette et al. (2004), and Vahter et al. (2000). The difference was due to the fewer fish and seafood consumption during the pregnancy, as recommended by the US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA). Both agencies recommend to avoid the consumption of certain fish types that contained high mercury concentration, including shark, swordfish, king mackerel and tilefish (Razzaghi et al., 2014). Another possible reason for the lower MeHg concentration in pregnant women might be due to the hemodilution and other physiological changes that are related to pregnancy (Vahter et al., 2000), and could also be caused by the MeHg movement from the maternal blood to the cord blood (Kim et al., 2011).

On the other hand, the blood MeHg concentration reported in the present study was lower than results reported by Basu et al. (2014) in the Early Life Exposure in Mexico to Environmental Toxicant (ELEMENT) cohort study among trimester pregnant women in Mexico City. The cohort study found that 28.6 to 39.2% exceed the 3.5 µg/L across all the trimesters. Besides that, the study also found that high mercury exposure among pregnant women was because of high fish and seafood intakes. In Mexico, seafood was consumed nearly 7 times a month in about 700 to 800 grams each time, and canned tuna was the most popular item consumed. These findings showed that the fishes and seafood consumption in Mexico was approximately 2 times higher than the amount of seafood consumed by the other women of child-bearing age in America (442 grams/month; EPA, 2002).

In our study, the type of marine fish intake and maternal blood MeHg level in pregnant women was examined. The finding shows that the top five marine fishes that are mostly consumed by the respondents were Indian mackerel, torpedo scad, yellow-banded scad, Spanish mackerel and pomfret. This finding was in line with the previous study by Ahmad et al. (2015), who have identified the most preferred seafood among the Malaysian in Peninsular Malaysia, and they reported that the majority of respondents preferred Indian mackerel (70.9%), followed by yellowtail scad (26.2%), pomfret (22.6%) and tuna (21.8%), as compared to other types of seafood. Another similar study on Hg exposure via fish consumption was by Jeevanaraj et al. (2016), who conducted a study in Selangor and found that the most preferred marine fish species was the Indian mackerel (55%), followed by torpedo scad (31.6%), Indian scad (15.2%), yellow-banded scad (12%), Eastern little

tuna (11.7%) and Japanese threadfin bream (10.5%). This shows that most of the people in the Klang Valley would prefer the mackerel and scads species over the other fish species.

In this study, the consumption of all seafood types (i.e., prawn, squid, crab and cockles and marine fishes) were not significantly associated with the maternal blood MeHg concentration among the respondents. This may be due to the low consumption amount of seafood and marine fishes among respondents in this study. Most of the respondents in this study were found to consume seafood and fish of small size on a monthly and weekly basis. Besides that, the lower Hg accumulation may be due to the respondents' preference that mostly favoured marine fishes, therefore contributing to the relationship between fish and seafood consumption with the MeHg accumulation. The Hg concentration in muscles of fish species mostly consumed by the respondents in this study showed the values below the Malaysian Food Regulation 1985 and Joint FAO/WHO Expert Committee on Food Additives (JECFA) guideline limit of 0.5 mg/kg (Jeevanaraj et al., 2016; Ahmad et al., 2015; Hajeb et al., 2008).

The minimum concentration of MeHg found in this study was lower while the maximum concentration was higher, compared to the studies conducted in Korea and Portugal. No Malaysian study on the MeHg concentration in the blood sample was found. Apparently, in the context of community health, the prevalence of 11.2% that exceeded the guideline limit of 3.5 µg/L reflects that the harmful exposure of Hg through fish consumption is at an alarming stage among the consumers. Regardless of the susceptible group, the Hg exposure can affect any individuals in the population who had consumed food contaminated with Hg, especially fish and seafood.

The measured maternal blood MeHg showed that 11.2% of the respondents had accumulated MeHg concentration beyond the guideline limit of 3.5 µg/L. The prevalence of exceeding limit reflects the current exposure among pregnant women with non-occupational exposure, typically due to fish consumption. The women in the highly exposed group are a critical concern to the health regulatory authorities and government bodies. These policymakers need to regulate the policy and guideline on advisory consumption of fish-contained Hg and proposing a health surveillance program among pregnant women to protect them from the over-accumulation of Hg and MeHg in their body.

CONCLUSION

The method for detecting MeHg in the blood sample and the extraction and instrument method detection carried out in this study was effective and accurate. The results from the samples showed that this study population was possibly exposed to MeHg via ingestion route through fish consumption. Exposure to the toxic substance during pregnancy may harm the unborn baby and lead to neurotoxicity. Therefore, there is a pressing need for

further investigation and evaluation among the susceptible group, especially on their dietary intake and other possible sources of exposure, in order to plan for a subsequent risk management approach.

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