ORIGINAL ARTICLE

Blood Heavy Metals (Arsenic, Cadmium and Lead) Concentration and Genetic Polymorphism of Glutathione S-transferase Genes Among Adults in Coastal Area of Melaka, Malaysia

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ABSTRACT

Introduction: Heavy metals exposure through the consumption of seafood are an environmental issue of concern. Biomonitoring of whole blood heavy metals concentration give an insight on the total internal body burden. The level of heavy metals of an individual are interrelated to the genetic polymorphism of glutathione S-tranferases (GSTs). This study aims to determine the association between blood arsenic (BAs), blood cadmium (BCd) and blood lead (BPb) concentration as reported in previous report and polymorphic variants of GST genes. **Methods:** Sixty three adult respondents were recruited along the coastal area of Melaka through purposive sampling in February 2016. Blood samples were collected through venipuncture procedure by certified personnel in EDTA tubes. The inductively coupled plasma- mass spectrometry (ICP-MS) instrumentation was used to analyse the blood heavy metals concentration. DNA was extracted from the whole blood samples. The polymorphic variants of *GSTM1* and *GSTT1* were analysed by multiplex polymerase chain reaction (PCR) and polymorphic variants of *GSTP1* were analysed by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP). **Results:** The BAs, BCd and BPb median (IQR) concentrations detected were 0.076 (0.059 – 0.107), 1.204 (0.670 – 2.094) and 0.076 (0.038-0.138) (µg/L) respectively. The prevalence of genetic polymorphism in *GSTM1* and *GSTT1* of 63.5% and 38.1% were observed. The number of respondents who have both deleted genes for *GSTM1* and *GSTT1* was 17 (27%). The frequency of genetic polymorphism in *GSTP1* was 55.5%, in which 29 (46.0%) of the respondents were having the heterozygous (Ile/Val) polymorphism while the other 6 (9.5%) respondents were having the homozygous mutant (Val/Val). **Conclusion:** The polymorphic GST gene variations were not statistically linked to heavy metal concentrations. Respondents with the Val/Val genotype in *GSTP1* and both *GSTM1* and *GSTT1* deletions, had a greater median of BAs and BPb
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Keywords: Blood Arsenic, Blood Cadmium, Blood Lead, Coastal Community, GST Genes Polymorphism

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INTRODUCTION

The anthropogenic origins of heavy metals accumulation in aquatic livings include heavy metal migration through groundwater from industrial waste, agricultural, and urban runoff. These heavy metals are then sedimented underwater, where they are eventually absorbed by aquatic life and may reach the human body through polluted food sources (1-2). Heavy metals including mercury, cadmium, and lead, as well as polychlorinated biphenyls (PCBs), are highly poisonous compounds that accumulate in the tissues of marine creatures and are then transferred down the food chain to humans (3-5). Toxic heavy metals for instance As, Cd and Pb which accumulate through the food chain may results in negative effects to human health (5-7). According to a study conducted along the Straits of Melaka in 2012, low levels of arsenic were found in wet weigh samples of fish such as the Longtailed butterfly ray, Gray eel-catfish, Spanish mackerel, and prawns while traced of Cd was reported in every seafood sample that have been collected. (8). The trace elements may accumulate in the human body even if ingested in little amounts over time

(9). Lead, for example is cleared from soft tissues and blood after 1 to 2 months, and from the skeleton after years to decades (10). The half-life of cadmium depends on the period of exposure where long-term build up in the body resulted in long half-life of 30 years and recent exposure is cleared between three to four months (11).

Arsenic is associated with increased developmental disorder, cardiovascular and metabolic diseases (12). The chronic As exposure has been reported to cause As toxicity which will lead to skin, bladder and lung cancers (13). The acute exposure to Cd may cause nausea, vomiting, abdominal cramp, and diarrhea while chronic Cd exposure had toxic effects in various organs (14-15). Previous studies showed that low levels of Pb in the blood could lead to hypertension (16) and accumulation of Pb in human bodies can harm the functions of renal and liver (17).

Blood heavy metals concentrations are biological indicator of exposure to reflect the individual's total internal burden with long-term exposure which receives inputs from recent exogenous exposure and tissue compartments (18). The determination of BAs, BCd and BPb concentrations reflect the exposure to heavy metals (19-21). Previous studies by Mohd Noor et al. and Md Zulkifli et al. have reported trace of heavy metals in all of the blood samples tested among the coastal community (22-23).

The genetic variations are among the internal contributing factors to individual's susceptibility to heavy metals toxicity (24). The glutathione-S-transferase (GST) is the enzyme accountable for the heavy metals' detoxification. The detoxification processes of GST in heavy metal metabolism play a key role in cellular antioxidant defense mechanisms (25). Heavy metals build up in the human body can alter cellular function and the electrochemical process (26). They are taken up by the liver and bind to glutathione, which is released by the liver and is used to eliminate and detoxify toxin and heavy metals from the body (27).

Polymorphism in GST genes (*GSTM1*, *GSTP1* and *GSTT1*) may disrupt the normal cellular activities (27-28). Deletions (null genotype) of *GSTM1* and *GSTT1* are the result of a whole or partial gene loss, and they may have a decreased ability to eliminate carcinogenic substances (29). The *GSTP1* polymorphism at codon 105 (exon 5) is an adenosine-to-guanosine (A-G) transition that results in isoleucine being replaced by valine in *GSTP1*'s substrate-binding site (30). The 105 form of isoleucine has decreased catalytic activity against various carcinogenic compounds (29). *GSTP1* catalytic activity is altered substrate-dependently by the replacement of the more hydrophobic valine (31). Because *GSTP1* polymorphisms alter detoxifying activity, it has been proposed that they have a substantial role in cancer susceptibility in a population. The presence of polymorphism in *GSTP1* gene was found to be associated with increase BAs and BPb levels (32). The combined polymorphism of *GSTP1* with *GSTM1* and/or *GSTT1* deletion is reported to affect the cadmium toxicity in the body (28).

In a prior study by Mohd Noor et al. and Md Zulkifli et al., there were no significant correlations between blood heavy metals content and possible heavy metals accumulation variables including sociodemographic background, seafood consumption frequency, and respondents' smoking behaviours (22-23). In this study, other potential factors that contribute to heavy metals accumulation which was the polymorphism in GST genes (*GSTM1*, *GSTT1* and *GSTP1*) was investigated. Heavy metals exposures along with glutathione-S-transferases polymorphisms have been associated with disease outcomes (33-34). The study on the individuals' susceptibility to heavy metals through the genetic polymorphisms gives an insight on how the individual variation may correlate with blood heavy metals concentration.

MATERIALS AND METHODS

Study Subjects

A total of 63 respondents were purposively recruited from healthy Malay adults in coastal regions such as Alor Gajah, Melaka Tengah, and Jasin. According to the recent survey, 90.5 percent of respondents consume seafood on a daily basis, which may contribute to heavy metal accumulation in the body (22).

Blood Sampling

Volunteers were venipunctured to collect eight millilitres of whole blood in two 4 ml EDTA tubes for heavy metal blood content assessment and DNA genotyping by qualified professionals. Heavy metal blood concentration samples were maintained at -20°C and analysed within 2 days, whereas DNA genotyping blood samples were retained at -80°C until further investigation.

Blood Analysis

Heavy Metal Analysis

As previously reported, the whole blood sample was digested using a wet acid digestion technique modified from Yahya et al before being analysed with inductively coupled plasma-mass spectrometry (ICP-MS) equipment (ICP-MS 7500c; Agilent Technologies, USA) (22-23; 35).

Genetic Polymorphism Analysis

DNA samples were extracted from the whole blood using QIAamp DNA Mini Kit based on the protocol (QIAGEN, Germany). Genetic polymorphism of *GSTT1* and *GSTM1* were detected by using multiplex PCR based on procedures described by Safarinejad et al. (36) with a little modification. Albumin gene was included as the housekeeping gene. They were amplified in 10µL

Table I. Forward and Reverse Primers for Each Gene with Their Product Size

Gene	Primer	Primer Sequence	Product Size
GSTT1	*GSTT1*-2F	5′-TTC CTT ACT GGT CCT CAC ATC TC-3′	459 bp
	GSTT1-2R	5′-TCA CCG GAT CAT GGC CAG CA-3′	
GSTM1	*GSTM1-2F*	5′-GAA CTC CCT GAA AAG CTA AAG C-3′	219 bp
	GSTM1-2R	5′-GTT GGG CTC AAA TAT ACG GTG G-3′	
Albumin	Albumin gene- 1F	5′-ACA CAA CTG TGT TCA CTA GC-3′	350 bp
	Albumin gene- 1R	5′-CAA CTT CAT CCA CGT TCA CC-3′	
GSTP1	*GSTP1*-1F	′5-ACC CCA GGG CTC TAT GGG AA-3′	176 bp
	GSTP1- 1R	5′-TGA GGG CAC AAG AAG CCC CT- 3′	

reaction mixtures consisting of 1.5 µL of ultrapure water, 4.5 µL of 10X Premix, 0.5 µL of each 10uM forward and reverse primers, and 1 µL of 10ng extracted DNA. Table I showed the sequences for the primers. The reaction took place with initial denaturation at 94°C for 5 min and then 25 cycles as follows: 90 s at 94°C, 60 s at 62°C, 60 s at 72°C, and a final annealing and elongation for 5 min at 62°C and 7 min at 72°C respectively. The products were separated using 3.0% agarose gel and visualized under ultraviolet using transilluminator. The presence of albumin fragment indicated the accurate functioning of reaction and the absence of *GSTM1* and /or *GSTT1* PCR product corresponded to gene deletion or polymorphism.

The detection of *GSTP1* Ile105Val polymorphism was conducted through polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique by Gonul et al. (37) with a little modification. The fragment of 176 bp *GSTP1* gene was first amplified in a 10 µL reaction mixtures consisting of 2 µL of ultrapure water, 5 µL of 10X Premix, 1 µL of 10uM forward primer, 1 µL of 10uM reverse primer, and 1 µL of 10ng extracted DNA. Table 1 showed the sequences for the primers. The reaction took place with initial denaturation at 94°C for 5 min and then 30 cycles as follows: 30 s at 94°C, 30 s at 60°C and 30 s at 72°C, and a final annealing and elongation for 4 min at 60°C and 7 min at 72°C respectively. The PCR products were digested with BsmAI (NBE ,UK) which consisted of 10 µL PCR product, 2 µL of 1X NE buffer, 7.5 µL of ultrapure water and 0.5µL of BsmAI. The mixture was incubated at 65°C for 6 hours. The products were separated on a 5% agarose gel electrophoresis, visualized by novel juice staining under an ultraviolet illuminator, scanned and photographed. Individuals with a single fragment of 176-bp indicated the present of homozygous Ile/Ile (no polymorphism) while individuals with both 93- and 83-bp fragments indicated the present of homozygous Val/Val (polymorphism). The presence of three fragments corresponded to heterozygous Ile/Val individuals (polymorphism).

Statistical Analysis

The data obtained was statistically reviewed by SPSS version 22. The blood heavy metals concentration and genetic polymorphism of GST genes were described through descriptive analysis. The correlation between heavy metals concentration and genetic polymorphism in GST genes were assessed by Mann-Whitney U and Kruskal Wallis tests. Statistical significance was defined as a p value of less than 0.05.

ETHICS STATEMENT

The study protocol was authorised by the Universiti Putra Malaysia Ethics Committee for Research Involving Human Subjects (JKEUPM), and the study was carried out as planned (JKEUPM Reference number: UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2).

RESULTS

The statistical descriptive levels of blood heavy metals were presented in Figure 1. The median BAs, BCd and BPb concentration of respondents were 0.076 (0.059 – 0.107), 1.204 (0.670 – 2.094) and 0.076 (0.038-0.138) (µg/L) respectively. The prevalence of *GSTM1*, *GSTT1* and *GSTP1* polymorphism were shown in Table II. A

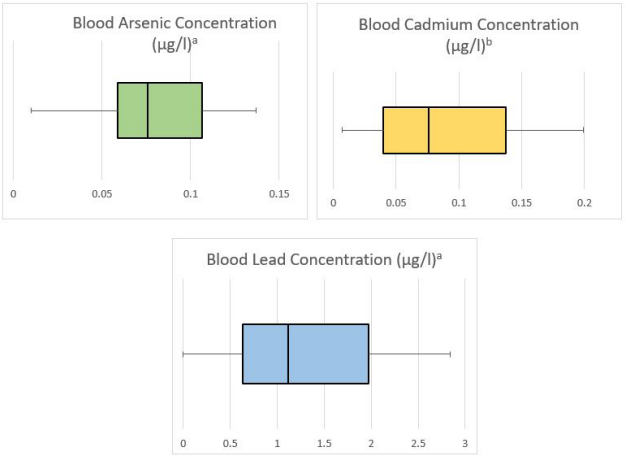

Figure 1: Respondents' Blood Heavy Metals Concentration (µg/L) (n=63)
a from previous report (22)
b from previous report (23)
reference value of arsenic in blood 1 µg/L (38)
reference value of lead in adult blood 50 µg/L (39)
reference value of cadmium in blood 0.315 µg/L (40)

percentage of 63.5% of the respondents were observed to have null genotype of *GSTM1* genes, 38.1% were having null genotype of *GSTT1* genes and 27% were having both deleted *GSTM1* and *GSTT1* genes. The frequency of polymorphism in *GSTP1* was 55.5% where heterozygous polymorphism (Ile/Val) was 46.0% and

Table II. Frequency of Polymorphism in GST Genes and Heavy Metals Concentration of Respondents (n=63)

GST Genes		Frequency		Median (IQR) (µg/L)		
		(N)	(%)	BAs	BCd	BPb
GSTM1	(+)	23	36.5	0.076 (0.049 - 0.085)	0.082 (0.064 – 0.224) [a]	1.037 (0.541 – 1.808)
	(-)	40	63.5	0.076 (0.063 - 0.123)	0.063 (0.035 – 0.122) [a]	1.623 (0.744 – 2.350)
GSTT1	(+)	39	61.9	0.075 (0.057 - 0.107)	0.074 (0.035 – 0.138) [a]	1.062 (0.702 – 2.614)
	(-)	24	38.1	0.081 (0.066 – 0.098)	0.079 (0.048 – 0.143) [a]	1.675 (0.649 – 1.832)
GSTM1 / GSTT1	(+) (+)	14	22.2	0.077 (0.056 – 0.108)	0.105 (0.054 – 0.243)	0.892 (0.504 – 1.883)
	(+) (-)	9	14.3	0.070 (0.048 – 0.084)	0.082 (0.069 – 0.187)	1.393 (0.589 – 1.937)
	(-) (+)	23	36.5	0.067 (0.056 – 0.107)	0.055 (0.035 – 0.102)	1.629 (0.838 – 1.876)
	(-) (-)	17	27.0	0.090 (0.072 – 0.159)	0.065 (0.039 – 0.131)	1.616 (0.708 – 3.078)
GSTP1	Ile/Ile	28	44.5	0.079 (0.058 – 0.105)	0.077 (0.053 – 0.172)	1.175 (0.520 – 1.784)
	Ile/Val	29	46.0	0.074 (0.057 – 0.102)	0.065 (0.038 – 0.129)	1.580 (0.793 – 2.610)
	Val/Val	6	9.5	0.109 (0.077 – 0.135)	0.065 (0.033 – 0.130)	1.683 (0.701 – 3.889)

homozygous mutant polymorphism (Val/Val) was 9.5%. The frequency of polymorphisms in GST genes and median of blood heavy metals concentration are summarized in Table III. The group with null genotype of *GSTT1* showed a slightly higher median BAs concentration of 0.081 (0.066 – 0.098) µg/L. Respondents with both deletion of *GSTM1* and *GSTT1* showed a higher median BAs concentration of 0.090 (0.072 – 0.159) µg/L. Respondents with Val/Val genotype has the highest median BAs concentration of 0.109 (0.077 – 0.135) µg/L.

Table III. Association of Heavy Metals Concentration with Polymorphism in GST Genes

GST Genes		BAs		BCd		BPb	
		Statistical Analysis	p-value	Statistical Analysis	p-value	Statistical Analysis	p-value
GSTM1	(+) (-)	U = 383.5	0.275	U = 334.0	0.072	U = 357.0	0.141
GSTT1	(+) (-)	U = 413.4	0.440	U = 431.5	0.605	U = 370.0	0.165
GSTM1 / GSTT1	(+) (+) (+) (-) (-) (+) (-)(-)	H = 6.488	0.090	H = 3.559	0.313	H = 3.406	0.333
GSTP1	Ile/Ile Ile/Val Val / Val	H = 3.576	0.167	H = 2.145	0.342	H = 3.335	0.189

Respondents with no null deletion in *GSTM1* had a higher median of BCd concentration which was 0.082 (0.064 – 0.224) µg/L compared to those who were polymorphic 0.063 (0.035 – 0.122) µg/L. The same trends were observed for the *GSTP1* gene where the median blood Cd concentration without polymorphism was higher with 0.077 (0.053 – 0.172) µg/L compared to polymorphic group with 0.065 (0.033 – 0.130) µg/L. The respondents with *GSTM1* polymorphism, *GSTT1* polymorphism and both deleted genes of *GSTM1* and *GSTT1* have a higher median BPb concentration of 1.623 (0.744 – 2.350), 1.675 (0.649 – 1.832) and 1.616 (0.708 – 3.078) µg/L respectively compared to the median of BPb concentration of total respondents. Respondents with Val/Val genotype has the highest median BPb concentration of 1.683 (0.701 – 3.889) µg/L.

The associations of blood heavy metals and polymorphism in GST genes are summarized in Table III. No statistically significant associations were observed between BAs, BCd and BPb concentration and genetic polymorphism of *GSTM1*, *GSTT1* and *GSTP1*.

DISCUSSION

In our previous report, the heavy metals concentrations observed among respondents have not exceeded the reference value where the BAs, BCd and BPb concentrations were significantly different from the standard value of heavy metals in blood; BAs was less than 10 g/L, BCd was less than 0.315 µg/L and BPb was less than 50 g/L respectively (22-23). The individuals in this study may be at a lesser risk of being negatively affected by recent heavy metal exposure due to the low amounts of heavy metals observed. Polymorphisms in glutathione-S-transferases influenced the individual ability to detoxify harmful intermediates and susceptibility to damage from oxidative stress (37). The *GSTT1*, *GSTM1*, and *GSTP1* polymorphisms were analysed to study the genetic susceptibility to heavy metals toxicity. More than half of the respondents were analysed with null polymorphism in their *GSTM1* gene. This is expected as the prevalence was in consistency with another study which determined the genotypic frequencies of GST genes in Kuala Lumpur that reported 66% of *GSTM1* polymorphism in Malay students (41) and 59.4% of *GSTM1* polymorphism among healthy control group (42).

Less than half of the respondents have the null genotype of *GSTT1* genes. This finding is expected, as previous study by Shahpudin et al. reported a prevalence of 32% (42). The prevalence was higher than a study in Malaysia by Mustafa et al. which reported 18% of *GSTT1* polymorphism in Malay students (41). The higher prevalence among respondents might be due to the imbalanced in the probability of the null alleles to be inherited to the subsequent generations (43).

The frequency of deletions in both *GSTM1* and *GSTT1* was slightly higher than the study by Mustafa et al. which reported a frequency of 18% (41). The difference in frequency of polymorphism might be due to younger age of respondents and no differentiation in terms of health status were made in current study which may affect the frequency of polymorphism in GSTM1 among the respondent in Melaka.

The frequency of *GSTP1* polymorphism was aligned to a study by Karen-Ng and colleagues which reported a prevalence of 58.6% of respondents were having Ile/Val and Val/Val genotypes (44). Furthermore, it was comparable to research by Shahpudin et al., who found that 40.6 percent of healthy controls had the Ile/Val gene and 3.1 percent had the Val/Val allele (42). A study in Selangor also reported a prevalence of 30.4% for Ile/Val genotype and 9.6% for Val/Val genotype (45).

No statistically significant associations were observed between blood heavy metals concentration and genetic polymorphism of GST genes in this study. This was consistent to a study in Jamaica where *GSTM1* gene tested was not significantly associated with level of whole BAs concentrations in the children (46). This was also in agreement with Khansakorn et al. which reported no association of *GSTT1* genotype with blood Pb levels (47) and a study among the riverside communities co-exposed to Pb in Amazon which reported no significant association between *GSTP1* with the BPb level (48). The lack of associations may be due to other genomes which are involved in heavy metals detoxification that were not assessed in our study and low exposure of heavy metals among the study community.

Current study found that the respondents with Val/Val genotype have the highest median BAs and BPb concentration. Similarly, Kaya-Akyddotuzlddotu et al. reported individuals with the Val/Val genotype showed lower urine arsenic levels but higher blood arsenic levels than Ile/Val and Ile/Ile genotypes (49). These data suggested that *GSTP1* enzyme activity affects blood and urine arsenic levels and produce inter-individual differences in blood. Changes in catalytic activity may cause a decrease in heavy metals-GSH conjugates and excretion, resulting in heavy metals accumulation in the blood. In contrast to our result, previous data by Rahbar et al. imply that typically developing Jamaican children with the *GSTP1* Val/Val genotype had a lower geometric mean blood arsenic content than those with Ile/Ile or Ile/Val due to a greater rate of arsenic detoxification from the blood (49).

In present study, respondents with both *GSTM1* and *GSTT1* deletion had higher median BAs and BPb concentrations. This might be explained by lower catalytic activity due to *GSTT1* and *GSTM1* gene deletion. However, the observation contradicts the findings of Rahbar et al. who found no statistically significant associations between blood arsenic concentrations and *GSTT1* or *GSTM1* genotypes in Jamaican children (46).

CONCLUSION

The results observed show that respondents with Val/Val genotype in GSTP1 and both deletion of *GSTM1* and *GSTT1* showed higher median BAs and BPb concentration. These findings revealed that difference in blood heavy metals concentrations is not completely associated to exposure in the environment but also to the genetic variation. We acknowledged the small sample size has resulted in the lack of statistical significance. Further analysis on a more specific enzyme responsible for heavy metals detoxification can be conducted to study into more details of their effects on blood heavy metals concentration.

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