UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF GSTM1 AND GSTT1 GENETIC POLYMORPHISM WITH BLOOD METHYLMERCURY LEVEL AND BIRTH OUTCOME AMONG FISHEATING URBAN PREGNANT WOMEN

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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ASSOCIATION OF *GSTM1* AND *GSTT1* GENETIC POLYMORPHISM WITH BLOOD METHYLMERCURY LEVEL AND BIRTH OUTCOME AMONG FISH-EATING URBAN PREGNANT WOMEN

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September 2020

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Methylmercury (MeHg) is the most toxic mercury species widely found in marine fish and is exposed to human through fish consumption. The exposure to MeHg during pregnancy has been associated with having significant effects on the central nervous system of developing fetus. MeHg might even affect the health of the newborns at the seemingly non-detrimental environmental exposure level. Marine fish consumption is known to be the main contributor to non-occupational MeHg exposure among the general population. MeHg elimination in human is related to the glutathione (GSH) detoxification system, in a bile mediated mechanism through the conjugation of *Glutathione S transferase* (GST) that transforms MeHg into stable form and eliminates through faeces. Both *glutathione-S-transferase- mu 1* (*GSTM1*) and *glutathione-S-transferase- theta 1* (*GSTT1*) genes are polymorphic in the human population, and the absences of these genes resulted in the loss of functional activity, thus leading to the increase of MeHg level in the body. Therefore, this prospective cohort study based in a Petaling district urban area of Selangor, was conducted to determine the maternal blood MeHg concentration level, to evaluate the association between the MeHg level to the *GSTM1* and *GSTT1* genes polymorphisms, to determine the frequency of marine fish consumption, and to obtain the birth outcome among the respondents. Pregnant women from the first trimester until the third trimester aged between 20 to 49 years old (N=215) who consumed marine fish participated in this survey and were included in the screening, interview, and blood sample collection processes. Five (5) mL of venous blood was sampled from each respondent and analyzed for the MeHg concentration by using liquid chromatography coupled with inductively coupled plasma mass spectrometry (LC-ICP-MS). The genotyping of *GSTM1* and *GSTT1* genes was performed using the polymerase chain reaction (PCR) method. Statistical analyses were performed using IBM SPSS to determine the maternal blood MeHg concentration, *GSTM1* and *GSTT1* genes polymorphism, the frequency of marine fish intake and the predictors of maternal blood MeHg concentration. The analysis showed that the median of

MeHg in maternal blood was 1.70 µg/L, 11.2% higher than the guideline limit of 3.5 µg/L Hg. The concentration of MeHg in the respondents ranged from 0.11 to 9.90 ug/L. The prevalence of *GSTM1* null and *GSTT1* null were 69% and 38%, respectively. For the combination genotypes of *GSTM1*-/*GSTT1*+, *GSTM1*+/*GSTT1*-, *GSTM1*-/*GSTT1*- and *GSTM1*+/*GSTT1*+, the frequency were 43%, 11%, 27% and 20%, respectively. The Chi-Squared (χ^2) test showed that there was no significant association between the *GSTM1* null and *GSTT1* null polymorphism with the maternal blood MeHg concentration of respondents (p = 0.088 and p= 0.077, respectively). However, those with the *GSTM1*+/*GSTT1*- genotype showed significant association with higher maternal blood MeHg concentration, as compared to other genotypes (AOR= 5.469, 95% CI = 2.03 – 14.73). The most significant contributor to the maternal blood MeHg was the *GSTM1*+/*GSTT1*- genotype (AOR=7.361, 95% CI = 1.68 – 32.16). The finding also shows that there was no association between the maternal blood MeHg concentration with the birth outcome. In conclusion, there appeared to be no evidence of an effect of fish consumption on maternal blood MeHg levels as well as no evidence of MeHg levels to birth outcome, however, there is a possibility of maternal blood MeHg exposure risk among those with *GSTT1* null genotype, as they may tend to retain MeHg in the body.

Keywords: MeHg exposure, genetic polymorphism, *GSTM1*, *GSTT1*, pregnant women, birth outcome

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HUBUNGKAIT POLIMORFISME GEN *GSTM1* DAN *GSTT1* DENGAN PARAS METILMERKURI DALAM DARAH DAN KESAN KELAHIRAN DALAM KALANGAN WANITA HAMIL YANG MEMAKAN IKAN

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Metilmerkuri adalah spesis merkuri yang paling toksik yang banyak ditemui dalam ikan laut dan menyumbang pendedahan kepada manusia melalui pemakanan ikan. Pendedahan kepada metilmerkuri semasa kehamilan telah dikaitkan dengan kesan siknifikan kepada sistem saraf pusat bayi yang sedang berkembang. Metilmerkuri mungkin memberikan kesan sampingan kepada kesihatan bayi walaupon ia kelihatan tidak berbahaya pada peringkat pendedahan persekitaran. Ikan laut telah dikaitkan sebagai penyumbang utama kepada pendedahan persekitaran metilmerkuri dalam kalangan populasi umum yang bukan dari pendedahan tempat kerja. Penyingkiran metilmerkuri dalam manusia berkait dengan sistem penyahtoksikan glutation (GSH) dalam hempedu yang diperantara oleh konjugasi *glutathione-S-transferase* (GST), yang mengubah metilmerkuri kepada bentuk yang stabil dan dikumuhkan melalui najis. Kedua-dua gen *glutathione-S-transferase- mu 1 (GSTM1*) dan *glutathione-S-transferase- theta 1* (*GSTT1*) adalah polimorfik dalam populasi manusia dan ketiadaan gen ini akan mengakibatkan kehilangan fungsi aktiviti dan meningkatkan tahap metilmerkuri dalam badan. Kajian ini dijalankan untuk menentukan tahap kepekatan metilmerkuri di dalam darah ibu dan menghubung kaitkan dengan polimorfisme genetik *GSTM1* dan *GSTT1* dan pemakanan ikan dalam kalangan peserta kajian. Kajian ini merupakan kajian kohot prospektif, dijalankan di kawasan bandar di Daerah Petaling, Selangor. Kajian ini dijalankan untuk menentukan hubungan tahap kepekatan metilmerkuri dalam darah, dan menilai hubungkait paras metilmerkuri terhadap gen polimorfisme *GSTM1* dan *GSTT1* ibu, kekerapan pemakanan ikan marin serta hasil kelahiran dalam kalangan responden. Wanita hamil dari trimester pertama hingga ketiga yang berumur antara 20 hingga 49 tahun (N=215) yang memakan ikan laut telah terlibat dalam kajian ini yang terdiri daripada saringan, temuduga dan persampelan darah. Sebanyak lima (5) mL darah vena telah di sampel daripada setiap responden dan di analisis bagi mengesan kepekatan metilmerkuri dengan menggunakan alat kromatografi cecair yang digabungkan secara speltrometri jisim

plasma (LC-ICP-MS). Kehadiran genotip *GSTM1* dan *GSTT1* telah dilakukan dengan menggunakan kaedah polymerase chain reaction (PCR). Analisis statistikal dilakukan dengan menggunakan peranti IBM SPSS. Kepekatan metilmerkuri, polimorfisme gen *GSTM1* dan *GSTT1*, kekerapan pemakanan ikan marin dan peramal kepekatan metilmerkuri telah dikenalpasti. Dapatan kajian menunjukkan median metilmerkuri dalam darah wanita hamil adalah 1.70 µg/L, dengan peratusan sebanyak 11.2% melebihi tahap kepekatan metilmerkuri, iaitu 3.5 µg/L merkuri dalam darah ibu. Kepekatan metilmerkuri dalam darah wanita hamil adalah di antara 0.11 – 9.90 µg/L. Peratusan *GSTM1* nul dan *GSTT1* nul adalah 69% dan 38% masing-masing. Analisis statistik chi-square (χ^2) menunjukkan *GSTM1* nul dan *GSTT1* nul tidak siknifikan dengan paras metilmerkuri dalam darah ($p > 0.05$). Gabungan genotip *GSTM1*-/*GSTT1*+, *GSTM1*+/*GSTT1*-, *GSTM1*-/*GSTT1*- dan *GSTM1*+/*GSTT1*+ adalah 43%, 11%, 27% dan 20% masing-masing. Individu yang bergenotip *GSTM1*+/*GSTT1*- menunjukkan hubungkait yang siknifikan dengan kepekatan metilmerkuri yang tinggi berbanding genotip yang lain. (AOR= 5.469, 95% CI = 2.03 – 14.73). Faktor yang paling siknifikan terhadap kepekatan metilmerkuri dalam darah adalah genotip *GSTM1*+/*GSTT1*- (AOR=7.361, 95% CI = 1.68 – 32.16). Dapatan kajian juga menunjukkan, tiada hubungkait di antara tahap kepekatan metilmerkuri dalam darah ibu dengan hasil kelahiran. Kesimpulannya, kajian ini menunjukkan bahawa tidak terdapat bukti terhadap kesan pengambilan ikan terhadap paras metilmerkuri dalam darah ibu serta paras metilmerkuri dengan hasil kelahiran, namun terdapat kebarangkalian risiko pendedahan metilmerkuri terhadap individu yang bergenotip *GSTM1* nul dan kecenderungan untuk mengumpul metilmerkuri di dalam badan.

Kata kunci: pendedahan metilmerkuri, polimorfisme genetik, *GSTM1*, *GSTT1*, wanita hamil, hasil kelahiran.

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LIST OF ABBREVIATIONS

MeHg	Methylmercury
Hg	Mercury
As	Arsenic
Pb	Lead
Cd	Cadmium
EPA	Environmental Protection Agency
RfD	References dose
WHO	World Health Organization
FAO	Food Administration Organization
DNA	Deoxyribonucleic acid
GST	*Glutathione S transferase*
GSTM1	*Glutathione S transferase (Mu) 1*
GSTT1	*Glutathione S transferase (Theta) 1*
NRC	National Research Council
MANS	Malaysian Adult Nutrition Survey
GEI	Genetic Environment Interaction
LC-ICP-MS	Liquid Chromatography Inductively Coupled Plasma Mass Spectrometry
PCR	Polymerase Chain Reaction
FFQ	Food Frequency Questionnaire
CMD	Congenital Minamata Disease
JECFA	Joint FAO/WHO Expert Committee and Food Additive
DHA	Docosahexaenoic Acid
SGA	Small Gestational Age

PTB	Preterm Birth
LBW	Low Birth Weight
CDC	Centre Diseases Control
BMI	Body Mass Index
MREC	Medical Review and Ethic Committee
JKEUPM	Jawatankuasa Etika Penyelidikan Melibatkan Manusia Univerisiti Putra Malaysia
NMRR	National Medical Research Registration
PPS	Probability Proportionate to Size
EDTA	Ethylenediaminetetraacetic Acid
SVD	Spontaneous Vaginal Delivery
TBE	Tris-borate-EDTA
HCL	Hydrochloric Acid
LOD	Limit of Detection
LOQ	Limit of Quantification
SRM	Standard Reference Material
NIST	National Institutes of Standard and Technology
SPSS	Statistical Package for Social Science
GM	Geometric Mean

CHAPTER 1

INTRODUCTION

1.1 Research Background

Mercury (Hg) is a component widely distributed in the environment and exists as the elemental, inorganic and organic forms (methylmercury, MeHg). The World Health Organisation (WHO) considers Hg as among the top 10 chemicals that may cause "major public health concern". This issue of ubiquitous Hg contamination globally led to the recent Minamata Convention on Mercury, a binding international treaty created to control anthropogenic Hg emission (United Nations Environment Programme, UNEP, 2019). A principal form of Hg that the general populations are mainly exposed to is the MeHg (Sheehan *et al.*, 2014), which is very toxic to both human and environmental health. The ability of MeHg to be retained in the animal tissues leads to biomagnification through the successive food chain in the aquatic system.

The highest concentration of MeHg is found in top predatory species. Many studies conducted showed that human MeHg exposure is mainly through seafood consumption (including freshwater and marine varieties, shellfish and marine mammals) (Jeevanaraj *et al.*, 2016, Ahmad *et al.*, 2015, Anual *et al.*,2018). Therefore, the population with a high frequency of fishes consumption is at high risk for MeHg exposure. MeHg exposure to human comes almost exclusively from the consumption of fish or shellfish and can be measured by the quantification in blood and hair. One study conducted among the American population found that 80 to 90% of mercury found in the blood specimen samples was MeHg (Wells *et al.*, 2016).

Pregnant women require a healthy balanced diet with good nutritional status to ensure the proper health and development of the fetus. The correct intake of nutrients from all food components are needed to ensure that the fetus can develop well. Protein is one of the food components that is essential during pregnancy. The source of protein can be varied, including from seafood such as fish. As mentioned above, seafood, including fish, is the originated source of MeHg exposure in human. Therefore, a fetus with developing brain is one of the high-risk groups for MeHg exposure, due to their increased susceptibility to environmental pollution. MeHg can be transferred to the fetus *via* the placenta, and to the offspring *via* breast milk (Iwai-Shimada *et al.*, 2015; Rebelo and Caldas, 2016; Valent *et al.*, 2011).

The effect of MeHg to the maternal blood and the birth outcome have shown conflicting results, as some studies reported that there is an inverse association between the birth weight and MeHg level, while some studies reported no association (Lee *et al.*, 2010; Murcia *et al.*, 2016; Vigeh *et al.*, 2018). Exposure to the environmental toxins was a factor that is directly contributed to the 3% of neurodevelopmental disorder, while up to 25% would be contributed to gene-environment interactions (NRC, 2000). The other effects of MeHg are the deficit in memory, attention and language tests, as well as causing permanent damage to the developing brain. Hence, MeHg exposure in pregnant women is a source of concern (NRC, 2000).

Some studies reported that the effect of low-level MeHg exposure could be detected with the appropriate behavioural (in animals) or psychometric (in humans) evaluations. The psychometric evaluation is related to the psychological assessments such as the skills and knowledge, abilities, attitudes, personality traits and educational achievements (Newland *et al.*, 2015). The main effects are to the nervous system upon short-term exposure, whereas the kidneys are affected upon long-term exposure (Yunus *et al.*, 2020). The potential toxic effects of Hg include the damages to the kidney, reproductive systems, immune, hematologic, cardiovascular, respiratory systems and brain (Aldroobi *et al.*, 2013).

The association of genetic polymorphism with a heavy metal concentration in food is considered as a new topic that has been gaining the interest of the researchers. There are major interindividual differences in the capacity to metabolize and detoxify drugs and other xenobiotics (Johansson & Ingelman-Sundberg, 2011). *Glutathione-S-Transferase* (GST) genetic polymorphism has associated with an increased susceptibility of numerous forms of cancers and diseases those caused by toxic chemicals and drug (Dasari *et al.,* 2018)

Therefore, the purpose of this study is to examine the influences of (1) fish and seafood consumption and (2) *GSTM1* and *GSTT1* polymorphism to the maternal blood MeHg concentration level of an individual. In addition, this study also aims to determine the effects to the birth outcome (such as birth weight, gestational age at delivery, head circumference, birth length, intrapartum complication, Apgar score and birth defect) to the MeHg exposure level in maternal blood.

1.2 Problem Statement

Currently, the detrimental health effects of chronic and low dose heavy metals exposures, as well as the health effects in sensitive populations, have drawn a special attention. According to the 2013 Priority List of Hazardous Substances and as presented by the Agency for Toxic Substances and Disease Registry (ATSDR), Hg is the third-highest ranked heavy metals after arsenic (As) and lead (Pb) (Kim *et al.*, 2015). In Malaysia, a few conducted studies to determine the level of Hg in

human population, aquatic organisms and environmental media reported that the anthropogenic activities were the environmental toxicant source in human, while the exposure to MeHg is through the ingestion of contaminated fish from the aquatic ecosystem. (Jeevanaraj *et al.*, 2016; Ahmad *et al.*, 2015; Hajeb *et al.*, 2010; Alina *et al.*, 2012).

Malaysia is a coastal state with shores being washed by the Straits of Malacca, along the east and west coasts of Peninsular Malaysia, the South China Sea, as well as the coasts of Sabah and Sarawak (Hajeb *et al.*, 2012). The Straits of Malacca is subjected to a great variety of pollutants due to its strategic location as a major international shipping lane, and the concentration of its agriculture, industry, and urbanization that predominate on the west Peninsular Malaysian coast (Hajeb *et al.*, 2012). However, the environment was threatened by the advancing development and uncontrolled human activities.

Ahmed *et al.* (2011) reported that fishes consumption increased from 49 kg per capita to 53 kg per capita in years 2000 to 2005, while the Ministry of Health Malaysia (2010) reported that Malaysian consume an estimated 59 to 62.5 kg of marine fishes every year and this amount is increasing over the years. In addition, a finding from the Malaysian Adult Nutrition Survey (MANS) 2014 found that 29.4% of Malaysian population consumed marine fishes with approximately 169.6 g/day portion intake (Noraida *et al.*, 2018). Among the fishes consumption reported above, there are a few fish species that exceeded the dose recommended by the FAO/WHO, Malaysia Food Act 1983 and Malaysian Food Regulation 1985. FAO/WHO has set the maximum allowed level of MeHg in all fishes (except predatory fishes) as 0.5 mg/kg, while the level was set as 1 mg/kg of MeHg for predatory fishes (such as shark, tuna, swordfish, pike and etc.). This recommended level is similar with the recommendation by the Malaysia Food Act and Regulation 1985.

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 exceeding the FAO/WHO recommendation of 0.5 mg/kg, with the maximum concentration of 0.90 mg/kg was reported in golden snapper (Jeevanaraj *et al.*, 2016). The demersal fish of higher trophic with increased weight accumulated more Hg compared to pelagic, lower trophic and smaller fish species.

Besides that, one study of the Hg exposure level from fish consumption found in the hair that was conducted in Petaling, and Hulu Langat District among women at reproductive age showed that 40.9% of them exceeded the recommended dose at 0.1 µg/g (Jeevanaraj *et al.*, 2015). These findings propel the current study to focus on the Petaling District to examine the MeHg concentration level from fish

consumption. Furthermore, the genetic polymorphism was added in this study variable to determine the genetic susceptibility of different individuals to detoxify the MeHg retained in the body.

Pregnant women need a healthy diet during their pregnancy term. Fish is one of the most important sources of protein and omega-3 fatty acid, which are essential for fetal visual and neurological development. However, studies showed that fish and seafood consumptions are the main sources of MeHg exposure (Marques *et al.*, 2014). Hg and other heavy metals in a woman's body can be transferred to the child *via* the placenta during pregnancy and *via* the breast milk during lactation (Iwai-shimada *et al.*, 2015). A study also demonstrated that a sub-population with concentrations of Hg near the Environmental Protection Agency (EPA) reference dose (RfD) showed adverse effects as the children were found to be deficit in various neurodevelopment tests (Sagiv *et al.*, 2012).

MeHg can be eliminated from the human body through the glutathione (GSH) detoxification system in the bile, such by using the enzyme *glutathione-S-transferase* (GSTs). The GST family comprises of several genes, and many of them are polymorphic in human (Kiendrebeogo *et al.,* 2019, Gara *et al.,* 2010). Epidemiological studies have found that some polymorphisms in GSH-related genes are associated with MeHg metabolism (Custodio *et al.*, 2004; Gundacker *et al.*, 2009). *GSTM1* and *GSTT1* genes that were deleted in the alleles also shown the elimination of the enzyme activity. Since MeHg detoxification from the human body occurs *via* GSH conjugation, polymorphism in GSH-producing or GSH-conjugating enzymes could alter the rate of MeHg detoxification.

Regrettably, to date, there is no systematic study that focused on these groups of the population, nor is there any guidelines that were developed to reduce their exposure to MeHg. Hence, an assessment of exposure to both MeHg and inorganic Hg, or the total Hg is essential for this population group along with the establishment of fishes consumption advisory guidelines to protect the mothers and the future progenies. The study of genetic polymorphism and the exposure to MeHg *via* fishes intake has gained interest among the researchers, due to the potential of these genetic polymorphisms in modifying and metabolising MeHg in the human body.

These findings proved that the allele differences might probably exist in one or more human genes, thus will affect the susceptibility to heavy metal toxicity. Besides that, the impact of MeHg exposure has also been considered not only to the mothers but also to their offspring. The birth outcome has been measured to determine any consequences or effects from the high exposure level of maternal blood MeHg concentration to the baby. This research is carried out to provide more knowledge regarding MeHg exposure and the genetic polymorphism in the susceptible group population.

1.3 Study Justification

This study focuses on the MeHg as the main exposure toxicant because it is a ubiquitous neurotoxicant. MeHg mostly present in the fish and fish as one of the sources of protein is eaten by a human. Thus, the human who eats fish is exposed to MeHg and this present a significant public health dilemma because fish consumption provides widely well-known nutritional benefits (Oken *et al.*, 2012). While eating fish during the pregnancy can have neuroprotective attributes that may counteract the MeHg effects (Sagiv *et al.*, 2014), but WHO still establish a safe guideline that limits fish MeHg dietary intake during pregnancy and during lactation (Åkesson & Caracalla, 2015). MeHg is a very poisonous Hg species that will bioaccumulate in exposed organism. Besides that, it has long half-life thus can cause long term and severe harm to the human body (Hong *et al.*, 2012). Hence, due to the bioavailability and bioaccumulation attributes of MeHg in the human body, it is very crucial to investigate the subsequent effects of the exposure level to human, especially in vulnerable groups such as pregnant women and newborn.

Pregnant women are the risky population group as they are more prone to the exposure of any hazardous substances that can give adverse health effects to themself and their fetus. Cumulative exposure to hazardous substance beginning at pregnancy and/or during the post-natal period can lead to early life neurodevelopmental challenges and the resulting disabilities (Marques *et al.*, 2014). Besides that, during pregnancy, the fetuses were linked to their mothers *via* the placenta; therefore, everything including toxic can pass through from mothers to the fetuses. In addition, pregnant women also need a balanced nutrient intake, including protein. Hence, pregnant women were known as a vulnerable group to MeHg exposure. Although the exposure to the low level of Hg does not cause any symptom to the mothers, the consequences highly impact the fetuses, as a developmental disorder due to the influences of MeHg to the cerebral nerves in fetuses had been reported (Hong *et al.*, 2012).

Human exposure to chemical contaminants can be characterised by examining biomarkers (Branco *et al.,* 2017; WHO 2011; Yusa *et al.,* 2012; Castro *et al.,* 2014). It has been proven by most researchers that blood and hair are the validated biomarkers to examine the MeHg intake that is correlated with seafood consumption in the general human population (Castano *et al.,* 2019; Yaginuma-Sakurai *et al.,* 2012). Blood has been used to study the presence of Hg and also indicate the timing of exposure. MeHg is readily absorbed through the gastrointestinal tract and is distributed throughout the body by the blood.

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 (NRC) (2000). After considering the effects of in utero MeHg exposure to child development from the Faroese, New Zealand 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), Silbernagell *et al.,* (2011) used this guideline limit to associate their maternal blood Hg and MeHg exposure levels. While Cusack *et al.,* (2017) suggested that, this guideline limit may be more relevant benchmark for comparison until an updated reference dose is determined.

To the best of our knowledge, there is no study conducted on MeHg exposure levels among pregnant women through fish consumption in Malaysia. The pregnant women population were chosen because this group is a susceptible group to xenobiotic exposure such as MeHg due to its highly toxic compound. Furthermore, previous study by Jeevanaraj *et al.,* (2015) who conducted a study on hair Hg exposure through fish consumption among women at reproductive age in Petaling and Hulu Langat District, Selangor reported that 40.9% and 49% of women exceeded the EPA RfD. From the finding of the study, women in urban area of Petaling District were exposed to the Hg from non-occupational exposure. Further to that, this present study was aimed to investigate the MeHg exposure among pregnant women, the susceptible group. The genetic polymorphism was added as the new knowledge of this study in investigated the MeHg exposure levels and also is the novelty in this study.

In this context, the genetic background of MeHg detoxification is vital because it may explain different degrees of inter-individual susceptibility. The elimination of MeHg in human is linked to the *glutathione-S-transferase* (GST) detoxification system in bile. Among the GST genes, *GSTM1* and *GSTT1* may be important, since the total deletion of either gene results in no enzyme and consequently no MeHg detoxification. Individuals with *GSTM1* and *GSTT1* homozygous deletion genotypes are generally, but not invariably, believed to be at high risk of developing cancer and other diseases (Yu *et al.,* 2016).

1.4 Research Objectives

1.4.1 General Objective

To evaluate and investigate the polymorphism of *GSTM1* and *GSTT1* genes, maternal blood MeHg concentration and the birth outcome (i.e. birth weight, gestational age at delivery, head circumference, mode of delivery, Apgar score and intrapartum complication and birth defect) among the pregnant women from the Petaling District, Selangor.

1.4.2 Specific Objectives

1. To identify the socio-demographic characteristics among the respondents.
2. To determine the level of MeHg in the maternal blood of respondents.
3. To determine the prevalence of species-specific marine fishes and seafood intake among the respondents.
4. To determine the prevalence of *GSTM1* and *GSTT1* polymorphism among the respondents.
5. To determine the prevalence of birth outcome (i.e. birth weight, gestational age at delivery, head circumference, mode of delivery, gender, Apgar score and intrapartum complication and birth defect) among the respondents.
6. To associate the species-specific marine fishes consumption with the maternal blood MeHg concentration among the respondents.
7. To associate the *GSTM1* and *GSTT1* polymorphism with the maternal blood MeHg concentration among the respondents.
8. To determine the potential factors (i.e. the respondent's characteristics, the respondent's portion intake of fish and seafood, species-specific marine fish consumption, as well as the *GSTM1* and *GSTT1* polymorphism) that significantly contribute to maternal blood MeHg concentration levels.
9. To determine the most significant predictor of maternal blood MeHg concentration among the respondents.
10. To associate the maternal blood MeHg concentration with the birth outcome among the respondents.

1.5 Research Hypothesis

1. There is a significant association between the species-specific marine fishes consumption with the maternal blood MeHg concentration among the respondents.
2. There is a significant association between the *GSTM1* and *GSTT1* polymorphism with maternal blood MeHg concentration among the respondents.
3. At least one of the factors (i.e. the respondent's characteristics, the respondent's portion intake of fish and seafood, the *GSTM1* and *GSTT1* polymorphism) is significantly related to the maternal blood MeHg concentration among the respondents.
4. There is a significant association between the maternal blood MeHg concentration with the birth outcome.

1.6 Significance of the Study

Human is exposed to heavy metal through many types of sources. However, human exposure to the MeHg *via* food has gained interest among the researchers. The study focusing on the effects of MeHg concentration in food such as fish that leads to genetic changing or modification can be considered as new, and the

fundamental studies on this area are rarely being conducted. The significances of conducting this study include to investigate the association between genetic polymorphism with the maternal blood MeHg concentration, to explore the genetic factors that can ascertain the sources of biomarker variability, and to narrow the gap between perceived and true health risks associated with the MeHg concentration *via* food consumption. MeHg that accumulate in the food like fish muscle may cause adverse health effects to the pregnant women, and the birth outcome hence will be further investigated in this study. The influences of genetic in the combination of others environmental factors, also known as the Gene-environment interaction (GEI), may act as additive risks factors in conjunction with particular gene allelic form (genetic polymorphism) to influence disease initiation and progression.

1.7 Definition of Term

1.7.1 Conceptual Definition

Maternal Blood MeHg Concentration

Maternal blood MeHg concentration is measured based on the detection of MeHg concentration in maternal blood during pregnancy. It is normally expressed in the µg/L unit. The USEPA has set the reference dose for the cord blood Hg concentration as 5.8 µg/L. However, Stern and Smith (2003) reported that the cord blood levels are on average 70% higher than maternal blood and thus the biomonitoring guideline for mercury in maternal blood should be 3.5 µg/L. The cord blood Hg relates to the existing MeHg exposure in the mother (Stern and Smith, 2003).

Genetic Polymorphism

Genetic polymorphism is defined as the trait inheritance that is controlled by a single genetic locus with two alleles, in which the least common allele should have a frequency of about 1% or greater. Genetic polymorphism is the difference in DNA sequences between the individuals, groups or populations with the types comprising of single nucleotide polymorphisms (SNPs), repetition, additions, deletions, and recombination. The existence of genetic polymorphism may be the result of chance process or may have been induced by external factors such as viruses or radiation (Ismail and Essawi, 2012).

Fish Intake

Fish intake is referred to as the dietary intake of different fish species based on daily consumption, including the serving and portion size of each consumption.

Pregnant Women

Pregnant women are the women currently carrying a developing embryo in their womb for nine months or so, with the journey associated and exposed to numerous health risks for both mother and developing child (WHO, 2018).

Potential Factors

Potential factors are the factors that have the possibilities to influence or affect the measurement outcomes. It is normally measured together with the other variables and is included in the analysis part.

Adverse Birth Outcome

The adverse birth outcome is the birth with unfavourable effects such as stillbirth, preterm birth, low birth weight, lower head circumference (microcephaly), neonatal death, congenital anomaly, and the Apgar score that is related to the neonatal diseases and high death rate (Weng *et al.*, 2014).

1.7.2 Operational Definition

Maternal Blood MeHg Concentration

The maternal blood MeHg concentration was determined by using instrument analysis LC-ICP-MS in this study. The obtained value for the concentration was converted into µg/L. In this study, the RfD used was according to the guideline suggested by Stern and Smith (2003), with the value revised from USEPA recommended guideline of 5.8 µg/L Hg in the cord blood to 3.5 µg/L Hg in the maternal blood, after taking into consideration on the maternal Hg to cord blood Hg ratio concentration. This value was associated with the potential risk of learning disabilities in the children later on in life. Previous studies Basu *et al.,* (2014), Razzaghi *et al.,* (2014), Donohue *et al.,* (2018), used this guideline limit to associate their maternal blood Hg and MeHg exposure levels. While Cusack *et al.,* (2017) suggested that, this guideline limit may be more relevant benchmark for comparison until an updated reference dose is determined.

Genetic Polymorphism

GSTM1 and *GSTT1* are the genes of interest and are known as polymorphic in the human population (Lee *et al.*, 2010). *GSTM1* and *GSTT1* polymorphism were identified by using the PCR method and were detected as either presence (wild) or absence (null) in the respondents.

Fish Intake

Fish frequency intake was determined by using the validated Food Frequency Questionnaire (FFQ). For the purpose of this study, the common marine fish consumed together with the portion size and the number of serving were included in the questionnaire. The preference of the marine fish species was selected based on a pilot study conducted among the respondents from an Obstetrics and Gynaecology Clinic located in Hospital Serdang. The fish frequency intake was then analysed using statistical testing to observe the dietary fish intake among the respondents.

Pregnant Women

For the purpose of this study, pregnant women were selected from all the health clinics in the Petaling District based on the following inclusion criteria; age ranging from 20 to 49 years old, single pregnancy, not a vegan practitioner, and no history of chronic disease. In addition, the pregnant women recruited were at the pregnancy gestation of more than or equal to 12 weeks.

Potential Factors

The potential factors measured in this study were the factors that may contribute to the maternal blood MeHg exposure such as the respondent's characteristics (age, race, household income, estimation of fish expenditure, dental amalgam filling, the respondent's lifestyle (eat fish angled and frequency eat at restaurant or stall), seafood consumption (prawn, squid, crab and cockle), fish frequency intake (freshwater and marine) and the portion size of fish eaten, species-specific marine fish consumed and the *GSTM1* and *GSTT1* polymorphism.

Birth Outcome

The birth outcome measured in this study were birth weight, gestational age at delivery, head circumference, birth length, intrapartum complication, Apgar score and birth defect. The birth outcome was measured after the women have delivered the baby, and the birth data were recorded by the nurse. The data then was obtained from each mother to relate it to the maternal blood MeHg concentration.

1.8 Conceptual Framework

Prior literature was used to illustrate the conceptual framework, as shown in Figure 1.1. The aim of the research is to fill the gap in MeHg research among the susceptible population, i.e. the pregnant women group in Petaling District, Selangor.

In general, MeHg exposure can result from the consumption of aquatic organisms. The frequency of intake was the main important factor that can contribute towards the MeHg concentrations in human. Exposure to MeHg may originate from marine fishes and seafood consumption due to the high pollution from both industrial and anthropogenic activities. In this study, the exposure *via* fish consumption is a concern, as fishes were reported as containing MeHg and Hg many folds higher than that of shellfish and other processed seafood.

The factor that can predict the MeHg retention in the human body was the presence of *GSTM1* and *GSTT1* genes. This factor was taken into consideration since both genes were highly polymorphic in the human population, thus may play an imperative role in predicting exposure status and can be reflected as biomarkers of susceptibility. The genes were expressed as absence or presence in order to identify the polymorphism status in the individuals. The epidemiological study on genetic factor in determining the concentration levels and the individual's susceptibility had become the greatest concern among the researchers to provide further evidence on the gene-environment studies.

The biomarker was used to quantify the MeHg concentration in human *via* biological fluid and tissues such as the blood, urine and hair samples. The MeHg intake through ingestion route can be identified in these biomarkers, especially after long term exposure. In this study, the blood sample was taken as the biomarker to determine the MeHg concentrations and to identify the genotyping of genetic polymorphism. Moreover, blood was selected as the biological samples as the high concentration of MeHg will bound to the haemoglobin in red blood cells.

On the other hand, other potential factors that may influence the MeHg concentrations in blood were measured and considered as well. These factors are comprised of socio-demographic and socio-economic status (i.e. age, race, household family income, fish consumption expenditure), dental amalgam filling, lifestyle that includes usage of any whitening soap and creams, fishing hobby and the frequency of eating fish meals in the restaurants, seafood and fish frequency intake (i.e. freshwater and marine fish), as well as the portion and serving size of fish among the respondents.

The health effects of the MeHg retention in the mothers that can be passed down to the offspring were the ultimate aims and were determined through the birth outcome. The MeHg can cross from the placenta to the fetus *via* cord blood, with high concentration detected in the cord blood as compared to the maternal blood. The birth outcomes measured in this study were birth weight, gestational age, head circumference, birth length, Apgar score, intrapartum complication and birth defect. However, other confounders might affect the birth outcome, such as the background information (i.e. age), lifestyle and anthropometric measurement (BMI) of the mothers.

In this study, there were two dependent variables measured throughout the study. The fish frequency intake and genetic polymorphism were the independent variables to the maternal blood MeHg concentration, which was the dependent variable for the determination of MeHg burden in the respondent. Following this, the maternal blood MeHg concentration was an independent variable to the birth outcome status in determining the effects of maternal blood MeHg concentration on the offspring.

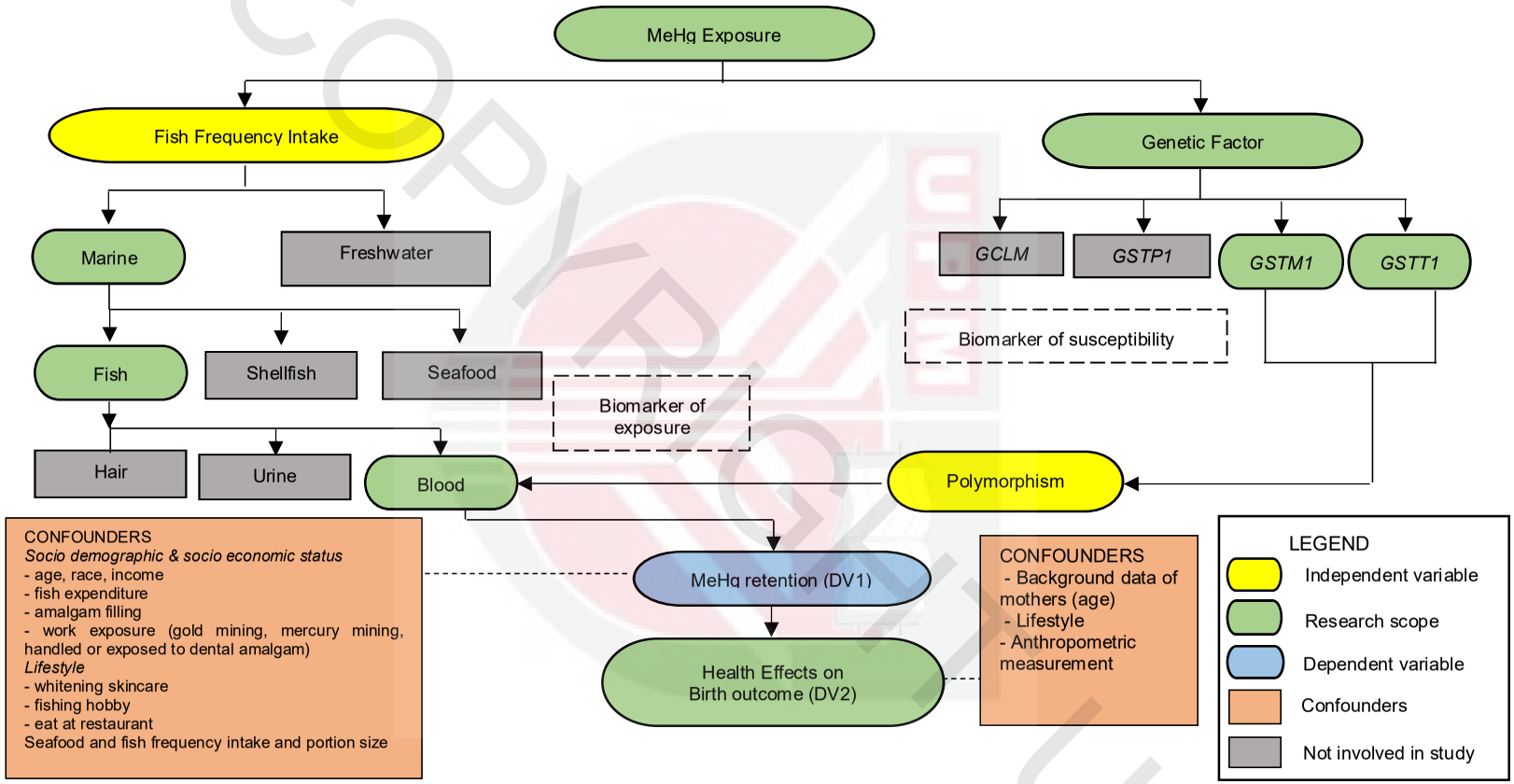

Figure 1.1 : Conceptual Framework of The Association Between Genetic Polymorphism of *GSTM1* and *GSTT1* Genes, with Blood Methyl Mercury Level, and Birth Outcome Among Pregnant Women in Petaling District, Selangor

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