UNIVERSITI PUTRA MALAYSIA

CADMIUM CONCENTRATION AND GENETIC POLYMORPHISM OF GSTM1, GSTP1 AND GSTT1 IN BLOOD SAMPLES OF RESPONDENTS FROM MELAKA, MALAYSIA

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By

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February 2018

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Eating seafood has become a major health concern for many people due to the present of the heavy metal especially cadmium (Cd) in that source of protein. Cd can accumulate in the body and disrupt the normal cellular processes which will eventually lead to organ damage. Due to the different of genetic composition of an individual, the amount of Cd accumulates in the body is different from each other even though they consume almost the same kind of food in everyday lives. This showed that the allele differences or specifically genetic polymorphism might probably exist in one or more human genes (*GSTM1*, *GSTT1* and *GSTP1*) which related to Cd detoxification. This study was conducted to determine the association between frequency intake of ten most consumed seafood and seafood products with blood Cd level and to determine the association between blood Cd level with genetic polymorphism of *GSTM1*, *GSTP1* and *GSTT1* among respondents in Malacca. A total of 403 respondents were recruited in this study and were given a set of questionnaire to gather information about seafood frequency intake and background information of potential exposure to Cd. Blood samples of 63 respondents were taken and analyzed by using ICP-MS. DNA from the blood samples were extracted and analyzed by using PCR and PCR RFLP to determine the presence and absent of polymorphisms in those genes. The most common seafood and its product that were frequently consumed by the respondents were shrimp paste (31.5%) followed by mackerel (13.6%), hardtail-scad (6.2%), flatfish (4.5%), fish ball (4.0%), fish crackers (2.2%), shrimp ball (2.2%), squid (2.2%), crab ball (2.2%), and wolf herring (2.0%). All the 63 blood samples showed the present of Cd with the median concentration of 0.076μg/L (IQR=0.1) and range between 0.007 μg/L to 1.284 μg/L. The median does not exceeded the permitted value stated by Agency for Toxic Substances and Disease Registry (ATSDR) which is 0.315 μg/L. There were 40 (63.5%) of the respondents having the *GSTM1* null genotype (polymorphism) while for *GSTT1* gene, there were 24 (38%) of them having *GSTT1* null genotype (polymorphism). For the *GSTP1* gene, there were in total of 35 (55.6%) of the

respondents having *GSTP1* polymorphism in their body. No association was observed between frequency intake of 10 mostly consumed seafood and seafood products with blood Cd level of the respondents. There was also no association found between blood Cd level and polymorphism of *GSTM1*, *GSTT1* and *GSTP1*. However, *GSTM1* and *GSTP1* genes showed some trends in which the respondents with no polymorphism (wildtype) were having higher median of blood Cd level compared to those who were polymorphic. Seafood and seafood products consumption were not significantly associated with the blood Cd level**.** Although blood Cd level was found to be not significantly associated with the polymorphism of those three genes, the trends observed during analysis showed that polymorphism of *GSTM1* and *GSTP1* may increased the efficiency of Cd to be secreted out of the body. Larger sample size is needed to further confirm the association between those two variables

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KEPEKATAN KADMIUM DAN POLIMORFISMA GENETIK *GSTM1*, *GSTP1* DAN *GSTT1* DALAM DARAH PENDUDUK DI MELAKA

Oleh

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Februari 2018

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Tabiat memakan makanan laut telah menjadi satu kebimbangan kesihatan yang utama bagi ramai orang kerana kandungan logam berat terutamanya kadmium (Cd) yang terdapat didalam sumber protein tersebut. Cd boleh terkumpul di dalam badan manusia dan mengganggu proses selular yang akhirnya akan membawa kepada kerosakan organ. Oleh kerana perbezaan komposisi genetik dalam setiap individu, jumlah Cd yang terkumpul dalam badan adalah berbeza antara satu sama lain walaupun mereka makan makanan yang hampir sama dalam kehidupan harian mereka. Ini menunjukkan bahawa perbezaan alel atau khususnya polimorfisma genetik mungkin wujud dalam satu atau lebih gen manusia yang berkaitan dengan detoksifikasi Cd. Kajian ini telah dijalankan untuk menentukan hubungkait antara kekerapan pengambilan sepuluh makanan laut dan produk makanan laut yang kerap di makan oleh responden dengan tahap kepekatan Cd dalam darah serta hubungkait antara tahap kepekatan Cd dalam darah dan polimorfisma genetik *GSTM1* , *GSTT1* dan *GSTP1* dalam sampel darah penduduk di Melaka. Seramai 403 responden dipilih untuk menyertai kajian ini dan telah diberikan satu set kaji selidik mengenai kekerapan pengambilan makanan laut serta produk makanan laut dan potensi pendedahan kepada Cd. Sejumlah 63 sampel darah responden telah diambil dan dianalisis dengan menggunakan ICP- MS. DNA dari sampel darah juga telah diambil dan dianalisis dengan menggunakan instrumen PCR untuk menentukan kehadiran dan ketiadaan polimorfisma dalam gen individu tersebut. Makanan laut dan produk makanan laut yang paling kerap di ambil oleh responden adalah belacan (31.5%) diikuti dengan ikan kembung (13.6%), ikan cencaru (6.2%), ikan sebelah (4.5%), bebola ikan (4.0%), keropok ikan (2.2%), bebola udang (2.2%), sotong (2.2%), bebola ketam (2.2%), dan ikan parang (2.0%). Kesemua 63 sampel darah menunjukkan kehadiran Cd dengan kepekatan median sebanyak 0.076µg/L (IQR=0.1) dan berjulat antara 0.007 µg/L sehingga 1.284 µg/L. Median kepekatan Cd ini tidak melebihi nilai yang dibenarkan oleh Agensi Bahan Toksik dan Pendaftaran Penyakit (ATSDR) iaitu 0.315 µg/L. Sejumlah 40 (63.5%) daripada responden mempunyai polimorfisme *GSTM1* manakala untuk gen *GSTT1*, terdapat

24 (38%) responden yang mempunyai polimorfisme *GSTT1*. Bagi gen *GSTP1* pula, terdapat 35 (55.6%) responden yang mempunyai polimorfisme *GSTP1* di dalam darah mereka. Tiada hubungkait antara antara sepuluh makanan laut dan produk makanan laut yang kerap di makan oleh responden dengan tahap kepekatan Cd dalam darah. Tiada juga hubungkait antara tahap kepekatan Cd dalam darah responden dengan polimorfisme *GSTM1*, *GSTT1* and *GSTP1*.

Walau bagaimanapun gen GSTM1 dan GSTP1 menunjukkan beberapa trend di mana responden yang tidak mempunyai polimorfisme (wildtype) mempunyai median tahap Cd darah yang lebih tinggi berbanding mereka yang polimorfik. Kekerapan pengambilan makanan laut dan produk makan laut tidak berkait dengan kepekatan Cd dalam darah. Kepekatan Cd dalam darah juga tidak berkait dengan polimorfisme *GSTM1*, *GSTT1* dan *GSTP1*. Walaupun tahap Cd dalam darah tidak berkait dengan polimorfisme ketiga-tiga gen tersebut secara signifikan, tetapi, trend yang dilihat semasa analisis menunjukkan bahawa polimorfisme *GSTM1* dan *GSTP1* dapat meningkatkan kecekapan sistem badan untuk mengeluarkan Cd keluar dari badan manusia. Sampel yang lebih besar diperlukan untuk mengkaji hubungkait antara dua pembolehubah dengan lebih lanjut.

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I certify that a Thesis Examination Committee has met on 2 February 2018 to conduct the final examination of Hamizah binti Md Zulkifli on her thesis entitled "Cadmium Concentration and Genetic Polymorphism of *GSTM1*, *GST1* and *GSTP1* in Blood Samples of Respondents from Melaka, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Date: 29 OCT 2019

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I hereby confirm that:

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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Signature: ______________________
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CHAPTER 1

INTRODUCTION

1.1 Background

Cadmium (Cd) is listed as one of the most toxic metals that exist in the environment (Djokic *et al*., 2014). It is a natural element that is mainly found in the earth crust at a concentration of 0.1 to 0.5 ppm and usually found to be associated with the other metals such as zinc, lead and copper ores (ATSDR, 2012).

Cd can be found in food especially in seafood and crops. Cd in soils is famously known to be more mobile and easily absorbed into plant tissues compared to lead and mercury (Singh and McLaughlin, 1999). Because of the mobility, it can easily contaminate the crops and eventually accumulate in human body as they consume the crops. Besides that, this toxic heavy metals can also contaminate the marine organisms because of the soil erosion that may eventually drove the contaminated soil into the sea (Verge and Petit, 2009). This explained why seafood are greatly exposed to cadmium contamination and it might be harmful to humankind because seafood such as fishes is one of the important protein sources in our daily diet.

Alina and collegues in Straits of Malacca traced Cd in every seafood samples that have been collected (Alina *et al*., 2012). Although the concentration of Cd in these marine organisms is in low concentration, the effect of the seafood consumption by human might appeared in a long term duration. It has been proved that prolonged exposure to heavy metals might cause serious health effects in humans (Singh *et al*., 2011). This environmental pollutant might cause various serious diseases such as kidney and liver failure (Walter and Joshua, 2006). Cd which is considered as human carcinogens also can cause cardiovascular, nervous system, blood and bone diseases (Jarup, 2003). The toxicity of the Cd has been proven on organs such as kidney and liver (Satarug *et al*., 2003).

In human body, Cd is taken up by the liver. Then, it will bound to glutathione (GSH), a compound that is produced naturally by the liver which function to excrete and detoxify toxins and heavy metals out of our body (Khansakorn *et al*., 2012). The binding site of glutathione is catalyzed by Glutathione S-Tranferase genes (GSTs) which comprises of Alpha (*GSTA*), Mu (*GSTM*), Pi (*GSTP*), Theta (*GSTT*), Sigma (*GSTS*), Omega (*GSTO*) and Zeta (*GSTZ*). The three most intensively studied are *GSTM1*, *GSTP1* and *GSTT1* (Buchard *et al*., 2007). GSTs are present in plants, insects, yeast, bacteria, and most mammalian tissues especially in the liver, which plays a key role in detoxification process. These genes are responsible for the stable conjugation of GSH-toxins in order for the toxins to be excreted out of the body (Ballatori, 2002). Polymorphism in one of these genes will results in decreased and altered enzyme activity which will lead to the difficulties in the detoxification of the heavy metals (Autrup, 2000).

This study aimed to investigate the association between frequency intake of ten mostly consumed seafood and seafood products with blood Cd level and to determine the association between blood Cd level with genetic polymorphism of *GSTM1*, *GSTP1* and *GSTT1* among respondents in Malacca. Given that the metabolism and detoxification of Cd in human body is mediated by glutathione and GSTs are involved in the stable conjugation of GSH-Cd conjugates, it is reasonable to hypothesize that polymorphism in GSTs genes could result in the different concentration of Cd among individuals body.

1.2 Problem Statement

Today, seafood such as fish and seashells has become the main supply of protein besides meat and poultry. This explained why more studies on the safety of the local seafood has gain interest among the researchers in Malaysia. Varied levels of heavy metals in fish samples were reported from various collection sites in Malaysia (Hajep *et al*., 2009; Irwandi *et al*., 2009; Alina *et al*., 2012). Hasyimah and friends in 2011 found that the aount of Cd and Pb in fish samples fall well between the permissible limit for human consumption when compared to the Malaysian Food Regulation, 1985 (Nor Hasyimah *et al*., 2011). Crustaceans which includes cockles, crabs and prawn are greatly exposed to heavy metals especially Cd because of their habitat which is sediment where various kind of hazardous and toxic substances are accumulated (Alina *et al*., 2012). Although the amount of Cd were found higher in cockles, the concentration was below the permissable value stated by the Malaysian Food Act 1983 and are safe to be consumed by human (Sabarina *et al*., 2014). Even though the findings of the studies creates a health concern particularly in community, due to less visible effects, the problem is neglected. Cd are toxic to human being. When they accumulate in the body, they will disrupt the normal cellular processes which will lead to the organ damage (Toxicology Factsheet Series, 2009) and later can cause variety of health effects. Studies showed that in some places, the amount of Cd accumulates in the body of a community is different from each other even though they consume almost the same kind of food in everyday lives. This might be due to the genetic composition of a population is regularly altered by natural events such as mutations, natural selection, and also migrations (Ungherese *et al*., 2010). Thus it proved that the allele differences might probably exist in one or more human genes which related to Cd detoxification.

The exposure and the toxicity of the Cd to human body have led researchers to conduct this study. The accumulation of Cd in human might be affected by the certain genes presented in human body. This study provided more knowledge regarding Cd exposure and the genetic polymorphism of a population. Genetic polymorphism can be studied through a certain gene present in the DNA. In this research, DNA was extracted from the blood sample. The *GSTM1, GSTT1* and *GSTP1* polymorphism were determined in order to further study its relation to the Cd concentration in human body. The concentration of Cd accumulated in a person was also determined by using the blood sample from the respondents. The seafood frequency intake and blood Cd level was determined to see the association between those two variables. The polymorphism of the *GSTM1*, *GSTT1* and *GSTP1* were determined to see the association between the blood Cd level and the polymorphism in those genes. This study would provide a basic data for future genetic studies in Malaysia.

1.3 Study Justification

In most Asian countries, especially in Malaysia, Indonesia and Thailand, fish is taken as the main source of dish in their diet (Alina *et al.*, 2012). Therefore, heavy metal level in aquatic organisms especially fish has been intensively studied in recent years as these substance can accumulate in the food chain. Some of these heavy metal are essential to human body but some of them can harm the body. Cd is believe to be part of the heavy metal which can cause negative effects to the body (Alina *et al.*, 2012). Cd was chosen as the analysed element because it is one of the earliest heavy metals that was detected in food which is in Japan in 1950s (Kaneta *et al.*, 1986). A local study on the heavy metal concentration in seafood along the The Straits of Malacca found that there were present of Cd in each of the analysed sample and the highest concentration was found in cockles compared to the other heavy metals (Alina *et al.*, 2012). Furthermore Cd can cause problem on kidney function and has been statistically associated with an increased risk of cancer. Therefore Cd was chosen as the study element to provide more information about blood Cd level in the community and thus help to reduce the risk of the health effect that might rise in the later age.

Blood was used as the biological sample to determine the polymorphism of *GSTM1*, *GSTT1* and *GSTP1* in human body because it is more accurate as it gives high quality of DNA and also RNA of an individual compared to the other biological sample such as nails, hair and urine. Urine is also considered as a good biological sample but it does not considered as an ideal source of DNA due to the low concentration of nucleated cells present in the urine (Amy and Pamella, 2012). Therefore blood was used as the biological sample in this study instead of urine. Besides that, according to Wen Zhang and friends, (1997), human blood is a good biological indicator for Cd exposure and considered as the main stream organs of Cd toxicity (Paul *et al.*, 2012). The concentration of Cd in whole blood also has gain wide acceptance as the most useful tools for screening and diagnostic testing. Besides that, study on the Cd blood level in human could be made as a baseline data about the safety of the local seafood as food chain is one of the main sources of exposure to heavy metal besides the exposure from workplace (Annabi *et al.*, 2013).

The study location which is located along the coastal area of Malacca is located at The Straits of Malacca (*Selat Melaka*). In recent years, as a result of industrial growth and increase of human population along the coastal area, Straits of Malacca was subjected to a rapid load of organic and inorganic effluent. This is because Straits of Malacca is famous in the whole world for its shipping and trading activities. Therefore in every minutes, many ships will be anchored at Straits of Malacca. This might affect the sea population, because the ships continuously release the gas and waste product into the sea. The toxic chemical from the ship also will be released into the sea which is the habitat of source of protein for the residents of Malacca and also Malaysia. The health of residents might be affected as they mostly consumed seafood from there. Besides that, there are various industrial activities take place along the west region of Penisular Malaysia (Alina *et al*., 2012). Therefore, the study location was determined along the coast of Malacca because the residents were expected to consume Cd contaminated seafood in their daily diet.

1.4 Conceptual Framework

Figure 1.1 shows the conceptual framework of various exposure of Cd to human. There are a lot of heavy metals that exist in the environment. Human can be exposed to Cd either from environmental exposure, occupational exposure or dietary habits. In the environment, Cd occurs naturally and is mainly found in the earth crust and also has been widely dispersed into the environment via the forest fires and volcanoes eruption (Mohammad *et al*., 2014; ATSDR, 2008). This toxic heavy metal may enter the air, water and soil via runoff and leaching as well as man-made routes which includes the usage of the phosphate fertilizers and the presence in sewage sludge (ATSDR, 2012). Indirectly, the water source became contaminated with the Cd and get into contact with the human (Verge and Petit, 2009). Cd also can be found in the industry and are used for the nickel and cadmium batteries.

For non-smoking population, food supply is the main route of exposure to Cd (ATSDR, 2012). Human may be exposed to Cd via the ingestion of the food that they consumed in their daily diet. Seafood especially those live in the sediments which includes cockles, crabs and prawns are greatly exposed to Cd because of their habitat which is sediment where all of the hazardous and toxic substances accumulate (Alina *et al*., 2012). This phenomenon might be harmful to humankind because seafood such as fishes is one of the important protein sources in the daily diet. Since villagers that are living along the coastal area are expected to consume mostly seafood and seafood based product in their daily diet, it can be assumed that Cd are present in high concentration in their body due to the consumption of these seafood.

The exposure to Cd either through inhalation, ingestion or absorption may lead to two types of health effect which are acute and chronic health effect. Acute Cd exposure or acute Cd toxicity is the adverse effect of Cd that results from a single exposure or multiple exposures in a short period of time (ATSDR, 2012). Several acute health effect includes irritation of the stomach and intestine, vomiting, and nausea. On the other hand, chronic Cd exposure or chronic Cd toxicity is the continuous or repeated exposure of Cd over a long period of time. This type of exposure might lead to the carcinogenic health effect which includes liver cancer, skin cancer, bladder cancer and lung cancer (Mathilde *et al*., 2015). Continous exposure to Cd also might lead to chronic health effect which includes renal proximal tubular damage and decline in glomerular filtration rate or renal failure (Kim *et al*., 2015)

The exposure of Cd in human body can be measured by using biological samples which includes blood, hair, nails and urine. Blood was used as the biological sample because it is more accurate as it gives high quality of DNA and also RNA of an individual compared to the other biological sample such as nails, hair and urine. DNA was extracted from the human blood and amplified by using Polymerase Chain Reaction (PCR) to double the targeted gene which is gene encoded for the production of glutathione.

Glutathione or GSH is an important antioxidant in human, plant, animal and also fungi. Presence of higher level of GSH in body can increase the metal tolerance of an organism while lower level of GSH may results in an increase susceptibility to heavy metal toxicity. In the detoxification process of the Cd out of the body, GSH need the aids of glutathione-s-transferases (GSTs). GSTs is widely known for their ability to catalyze the conjugation between GSH and xenobiotic substrate and heavy metals for the purpose of detoxification. There are 6 frequently studied GSTs which include alpha (*GSTA*), mu (*GSTM*), pi (*GSTP*), theta (*GSTT*), sigma (*GSTS*) and kappa (*GSTK*). The presence of any defects or specifically genetic polymorphism in those genes may interfere the Cd detoxification process in the body.

In this study, DNA for genetic study was extracted from the blood of the respondents and were then amplified by Polymerase Chain Reaction (PCR). The products were analyzed on agarose gel and visualized by novel juice staining to determine the presence of the polymorphism in *GSTM1*, *GSTT1* and *GSTP1*.

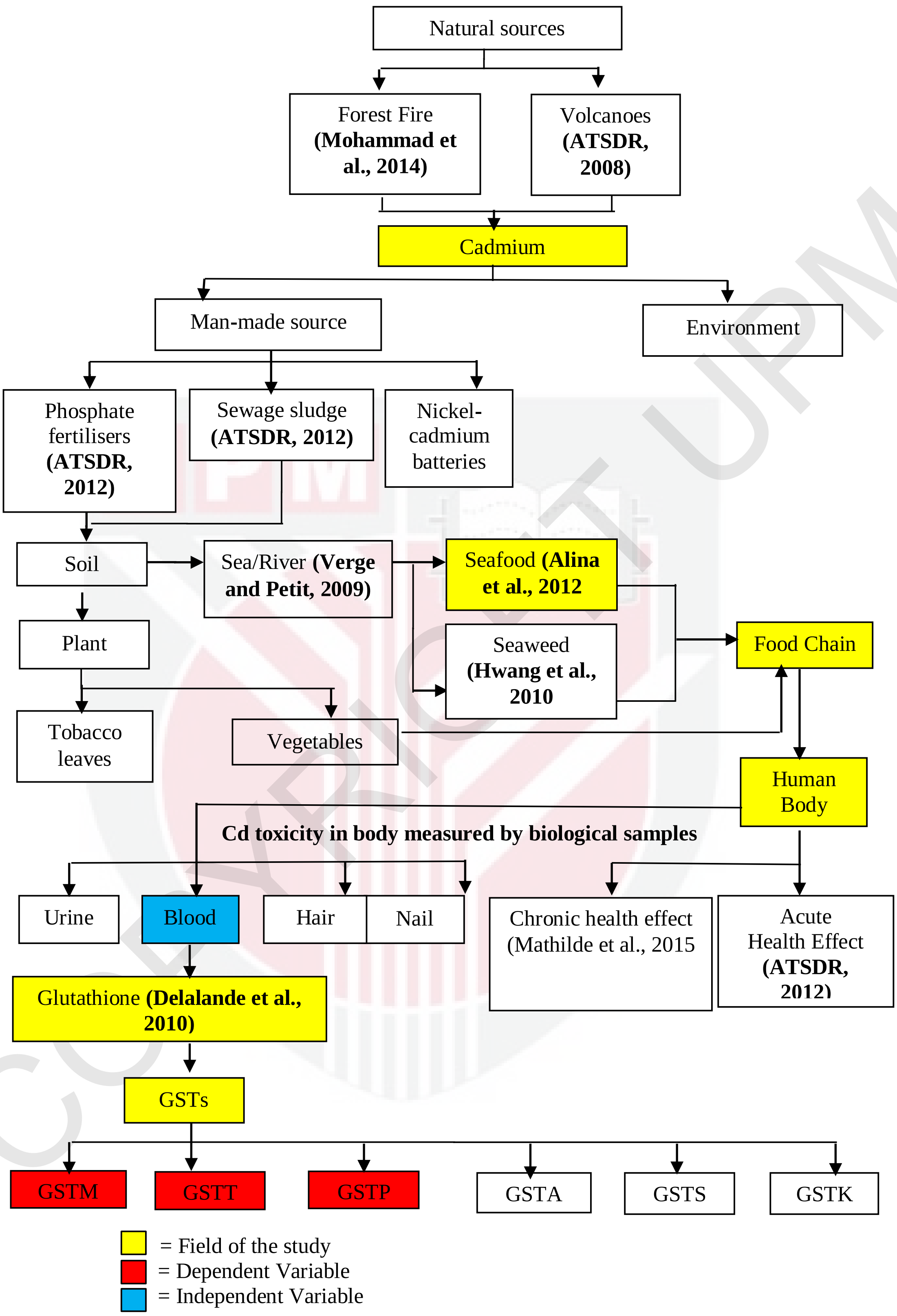

Figure 1.1 : Conceptual Framework of the Study

1.5 Definition of Terms

1.5.1 Conceptual Definition

1.5.1.1 Cadmium Level in Blood

Cd level in blood is the amount of Cd present in human body by using blood as the indicator. According to the Agency for Toxic Substances and Disease Registry (2012), the permitted Cd level in human blood in general population (≥1 year old) is 0.315 µg/L. Cd level in whole blood of a person whom not occupationally exposed generally range from about 0.0035 to 0.0089 mol/L for nonsmokers and 0.012 to 0.035 mol/L for smokers (Cadmium Public Health Guidance Note, 2002). Cd blood level of 0.089 mol/L are considered acceptable in workers with occupationally expose to this heavy metal. A person with occupationally expose to Cd by inhalation may have the Cd blood level up to 0.44 mol/L (Cadmium Public Health Guidance Note, 2002).

1.5.1.2 Food Frequency Intake

According to Agudo and Joint (2005), food frequency intake is the measurement of food intake that may be affected by differences in definition of groups of food. It is the report of the frequency of consumption and portion size of approximately 125 line items over a defined period of time. The Food Frequency Questionnaire (FFQ) is the most common dietary assessment tool used in large epidemiologic studies of diet and health.

1.5.1.3 Genetic Polymorphism

Polymorphism can be define as the ability to exist in several forms. While genetic polymorphism is the occurrence in the same population of two or more genetically determine phenotypes in such proportions that the rest of them cannot be maintained merely by recurrent mutation (Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, 2003). Genetic polymorphism promotes diversity within a population and usually persists over many generations.

1.5.2 Operational Definition

1.5.2.1 Cadmium Level in Blood

In this study, the Cd level in whole blood was determined by using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and presented in the unit of µg/L. According to Agency for Toxic Substance and Disease Registry (2012), the normal geometric blood Cd level in general population (≥1 year) is 0.315µg/L. Therefore the cut-off point for blood Cd level that is used for this study is 0.315 µg/L.

1.5.2.2 Food Frequency Intake

The food frequency intake of the respondents were determined by the Food Frequency Questionnaire (FFQ) which focusing into certain common seafood that is mostly consumed by Malaysians. In this study, the Food Frequency Questionnaire (FFQ) consist of list of food items that were consumed over a year. The respondents were interviewed by the researcher to recall the dietary intake of certain foods that they consumed over the year. The final intake was expressed in two distinct categories which are frequently consumed and not frequently consume.

1.5.2.3 Genetic Polymorphism

In this study the occurrence of genetic polymorphism were visualized by novel juice staining after the amplification reaction that were carried out by Polymerase Chain Reaction (PCR). For *GSTM1* and *GSTT1* genes, the polymorphism were detected by the absent of the PCR product of the respective genes (Sharma *et al*., 2014). These absent of the allele are called *GSTM1*- null genotype and *GSTT1*- null genotype. This assay does not distinguish between heterozygous or homozygous wild type genotypes. Genotyping of the *GSTP1* IIe105Val gene polymorphism was carried out via the Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RFLP) method (Buchard *et al*., 2007). This method was used for the detection of intraspecies as well as interspecies of variation.

1.6 Objectives

1.6.1 General Objective

To determine the relationship between Cd concentration and genetic polymorphism of *GSTM1, GSTP1* and *GSTT1* in blood sample among respondents in Malacca

1.6.2 Specific Objectives

1. To determine ten food that are most frequently consumed by the respondents
2. To determine ten seafood and seafood products that frequently consumed by the respondents
3. To determine the blood Cd level of the respondents
4. To determine the presence of genetic polymorphism of *GSTM1,GSTP1* and *GSTT1* in blood sample of the respondents
5. To determine the association between of ten seafood and seafood products that frequently consumed and blood Cd level of respondents
6. To determine the association between blood Cd level and other factors of exposure to Cd other than seafood and seafood product

7. To determine the association between blood Cd level and genetic polymorphism of *GSTM1*,*GSTP1* and *GSTT1* in blood sample of the respondents

1.7 Hypothesis

1. There is a significant association between ten seafood and seafood products that frequently consumed and blood Cd level of the respondents
2. There is a significant association between blood Cd level and genetic polymorphism of *GSTM1*,*GSTP1* and *GSTT1* in blood of respondents

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