UNIVERSITI PUTRA MALAYSIA

DISTRIBUTION OF GSTM1 AND GSTT1 POLYMORPHISMS AMONG CAUCASIANS AND THE THREE MAJOR ETHNIC GROUPS IN MALAYSIA

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Glutathione S-transferases (GSTs) are the vital Phase II enzymes to detoxify carcinogens and reactive oxygen species in the body, which encoded by GST mu class 1 (*GSTM1*) and GST theta class 1 (*GSTT1*) genes. Polymorphism in the genes affects the enzyme expression and increases the susceptibility to disease. *GSTM1* and *GSTT1* deletions present in 50% and 20% of Caucasian population, 54% and 37% in Africans, and 62% and 20% in Asians, respectively, showing ethnic-dependent polymorphism of both genes. However, the genetic polymorphisms are less reported in Malaysia predominantly between three major ethnics; Malay, Indian and Chinese. In addition, the rate of GST polymorphisms in Malaysia has not been precisely compared with Caucasian population using the standard genome reference, European Collection of Cell Culture (ECACC). Therefore, this research was to determine the distribution of *GSTM1* and *GSTT1* polymorphisms among three different ethnic groups in Malaysia and Caucasians. One to three milliliters of peripheral blood were collected from 518 individuals comprised of 262 Malays, 151 Chinese and 105 Indians. DNA was extracted using QIAamp DNA blood mini kit and quantified using NanoDrop™ Lite Spectrophotometry. Meanwhile, 192 DNA samples (ECACC HRC-1 and HRC-2) were commercially bought to represent Caucasians group. All DNA samples proceed with multiplex PCR to determine *GSTM1* and *GSTT1* deletion simultaneously in the presence of internal control, Albumin (*ALB*) gene. The genotypes were then validated using DNA sequencing. Chi Square test was carried out to compare the genotype frequencies among three Malaysian ethnic groups and between the whole study cohort in Malaysia and Caucasians at $p<0.05$ as significant difference level. This study recorded three categories of genotypes; *GSTM1* null, *GSTT1* null, and combined *GSTM1* and *GSTT1* null. There was a significant difference between the three Malaysian ethnic groups in all genotype categories ($p<0.001$). *GSTM1* null was found in 62.2% of

Malays, 48.3% of Chinese and 29.5% of Indians. As for *GSTT1*, the null frequency was higher among Chinese (47.7%) followed by Malays (39.7%), and lowest in Indians (9.5%) respondents. Meanwhile, the combined null genotypes were recorded in 26.0% of Malay, 21.2% of Chinese, and 2.9% of Indian. In overall, the whole study cohort in Malaysia and Caucasians were significantly different in the null genotype of *GSTT1* and combined genes ($p<0.001$). Malaysia has a higher null frequency of 35.9% for *GSTT1* and 19.9% for combined genes than Caucasians (16.7% for *GSTT1* and 8.9% for combined genes). The distributions of *GSTM1* in Malaysia are similar to those reported among Africans, Caucasians and Asians whereas the frequency of *GSTT1* null varies among those populations. In conclusion, Malaysia has a high rate of *GSTM1* and *GSTT1* null genotypes, and Malays showed the highest frequency among the three major ethnics. This study provides further evidence for ethnic variation in metabolism and disposition, and these genotype data will help future genetic studies on the GST polymorphisms in association with disease risks and drug effects in Malaysia.

Keywords : *GSTM1*, *GSTT1*, polymorphism, ethnics, Malaysia

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TABURAN POLIMORFISMA *GSTM1* DAN *GSTT1* DALAM KALANGAN ORANG BERKULIT PUTIH DAN TIGA KUMPULAN ETNIK UTAMA DI MALAYSIA

Oleh

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Glutathion S-transferases (GSTs) adalah enzim Fasa II yang penting untuk menyahtoksifikasi karsinogen dan metabolit yang sangat reaktif dalam tubuh. GST kelas mu 1 (*GSTM1*) dan GST theta 1 (*GSTT1*) adalah dua gen yang mengekod enzim tersebut. Polimorfisma dalam kedua-dua gen tersebut mempengaruhi ekspresi enzim itu dan meningkatkan kerentanan terhadap penyakit. Kehilangan *GSTM1* dan *GSTT1* masing-masing berlaku dalam 50% dan 20% orang berkulit putih, 54% dan 37% orang Afrika, dan 62% dan 20% orang Asia, menunjukkan kebergantungan polimorfisma kepada etnik oleh kedua-dua gen. Walau bagaimanapun, polimorfisma genetik itu kurang dilaporkan di Malaysia terutamanya di antara tiga etnik utama; Melayu, Cina, dan India. Tambahan lagi, kadar polimorfisma gen di Malaysia masih tidak dibezakan dengan populasi orang berkulit putih secara terperinci menggunakan ukuran rujukan genom, *European Collection of Cell Culture*. Oleh itu, kajian ini bertujuan menentukan taburan polimorfisma *GSTM1* dan *GSTT1* dalam kalangan tiga kumpulan etnik di Malaysia dan orang-orang berkulit putih. Satu hingga tiga mililiter darah periferi telah dikumpulkan daripada 518 inidividu yang terdiri daripada 262 orang Melayu, 151 orang Cina, dan 105 orang India. DNA telah diekstrak menggunakan kit mini darah QIAamp DNA dan diukur menggunakan NanoDrop™ Lite Spectrophotometry. Manakala, 192 sampel DNA (ECACC HRC-1 dan HRC-2) telah dibeli secara komersial bagi mewakili orang-orang berkulit putih. Kesemua sampel DNA diteruskan untuk tindak balas multiplex PCR bagi menentukan kehilangan *GSTM1* dan *GSTT1* secara serentak dengan kehadiran kawalan dalaman, gen Albumin (*ALB*). Kemudian, genotip-genotip tersebut telah disahkan melalui analisis penjujukan DNA. Ujian Chi Square telah dijalankan untuk membandingkan frekuensi genotip antara ketiga-tiga kumpulan etnik di Malaysia dan antara keseluruhan kohort kajian di Malaysia dan orang-orang berkulit putih di tahap $p<0.05$ sebagai tahap perbezaan yang ketara. Kajian ini merekodkan tiga

kategori genotip; nul *GSTM1*, nul *GSTT1*, and gabungan nul *GSTM1* dan *GSTT1*. Terdapat perbezaan yang ketara antara ketiga-tiga kumpulan etnik Malaysia dalam semua kategori genotip ($p<0.001$). *GSTM1* nul hadir dalam 62.2% orang Melayu, 48.3% orang Cina, dan 29.5% orang India. Bagi *GSTT1*, frekuensi nul gen tersebut lebih tinggi dalam kalangan responden Cina (47.7%) diikuti oleh Melayu (39.7%) dan paling rendah India (9.5%). Sementara itu, gabungan genotip nul direkodkan dalam 26.0% dari etnik Melayu, 21.2% etnik Cina, dan 2.9% etnik India. Secara keseluruhan, keseluruhan kohort kajian di Malaysia dan orang-orang berkulit putih hanya berbeza dalam genotip nul bagi *GSTT1* dan gabungan gen ($p <0.001$). Malaysia mempunyai frekuensi nul yang lebih tinggi iaitu 35.9% bagi *GSTT1* dan 19.9% bagi gabungan gen berbanding orang-orang berkulit putih (16.7% bagi *GSTT1* dan 8.9% bagi gabungan gen). Pengagihan genotip *GSTM1* di Malaysia adalah serupa dengan yang dilaporkan dalam kalangan orang-orang Afrika, berkulit putih dan Asia manakala kekerapan nul *GSTT1* adalah berbeza dalam kalangan populasi tersebut. Kesimpulannya, Malaysia mempunyai kadar genotip nul *GSTM1* dan *GSTT1* yang tinggi, dan orang-orang Melayu menunjukkan kekerapan tertinggi dalam kalangan tiga etnik utama itu. Kajian ini membuktikan secara lebih lanjut mengenai variasi etnik dalam metabolisme dan pelupusan, dan data genotip ini akan membantu kajian genetik masa depan mengenai polimorfisme GST bersamaan dengan risiko penyakit dan kesan ubat-ubatan (dadah) dalam populasi Malaysia.

Kata kunci : *GSTM1*, *GSTT1*, polimorfisma, etnik, Malaysia

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I certify that a Thesis Examination Committee has met on 3 April 2018 to conduct the final examination of Wan Nooremira binti Wan Rashidi on her thesis entitled "Distribution of *GSTM1* and *GSTT1* Polymorphisms Among Caucasians and the Three Major Ethnic Groups in 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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LIST OF ABBREVIATIONS

DME	Drug metabolizing enzymes
GSTs	Glutathione S-transferases
GSH	Glutathione
GSTT	Glutathione S-transferase class theta
GSTM	Glutathione S-transferase class mu
GSTM1	Glutathione S-transferases class mu 1
GSTT1	Glutathione S-transferases class theta 1
CYP	Cytochrome P-450 monooxygenase system
UGTs	UDP-glucuronosyltransferases
SULTs	Sulfotransferases
MAPEG	Membrane-associated proteins in eicosanoid and glutathione metabolism
ECACC	European Collection of Cell Culture
HRC	Human Random Control
SPSS	Statistical Package for the Social Sciences
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
TBE	Tris-Borate-EDTA
Bp	Base pair
NCBI	National Center for Biotechnology Information

CHAPTER 1

INTRODUCTION

1.1 Research Background

Glutathione s-transferases (GSTs) are the enzymatic system involved in cellular defense against genotoxic molecular. The catalytic activity of the enzyme overcomes the toxicity of environmental carcinogens, pharmaceutical drugs and other xenobiotics (Josephy & Mannervik, 2006). Phase 1 xenobiotic enzymes activate the compound through a reaction with oxygen catalyzed by cytochrome P450s (CYPs) and may produce electrophile and genotoxic molecules. These toxic compounds attack the cellular DNA and important functional proteins to cause cellular damage (Josephy & Mannervik, 2006). Besides xenobiotics, natural cell constituents, in particular lipids, can also give rise to genotoxic molecules in our body such as activated alkenes, epoxides and organic hydroperoxides by the reaction of oxidative and nitrosative stress (Mangialasche et al., 2009). Prior to these events, Phase 2 detoxification enzymes play a vital role in the biotransformation of highly reactive products or compounds to a non-toxic and water soluble form (Omiecinski et al., 2011). The major group of the detoxification enzymes is represented by glutathione S-transferases (GSTs) superfamily that detoxifies toxic substances or chemical carcinogens in the cell and subsequently will protect the DNA inside the cell from damage. GSTs conjugate the activated metabolites with glutathione and generate relatively inactive and more hydrophilic products that are soluble enough to be excreted from the body (Yadav et al., 2011).

GSTs are the supergene family of drug-metabolizing enzymes and the most important component in carcinogen metabolism due to their high distributions in human body compared to other Phase 2 metabolizing enzymes. GSTs possess the enzymatic activity of detoxifying carcinogens, pharmaceutical drugs, and environmental pollutants (Hayes & Pulford, 1995). Hence, the biological consequences of low capacity of detoxification system are usually related to the defective GSTs activity. The centers of attention in GST gene studies are Mu class (*GSTM1)* and Theta class (*GSTT1)* isoforms. The expression and activity of the enzymes will be altered when both genes revolve in mutations (Josephy, 2010).

Previous investigations reported that *GSTM1* and *GSTT1* are the only genes among all GST genes, which unable to be transcripted into its protein due to deletion in the gene locus (Shokeer & Mannervik, 2010). *GSTM1* was the earliest GST gene found to have the entire deletion of the gene at chromosome 1p13.3, followed by the discovery of *GSTT1* null allele resulted from the deletion of the entire gene at chromosome 22q11.2 (Warholm et al., 1980; Warholm, et al., 1983; Pemble et al., 1994). An absence of the gene will cause no production of GST enzymes and result in lack of detoxification system (Parl, 2005). A number of studies proved such

particular gene deletions can influence the occurrence of diseases such as colorectal, prostate, nasopharyngeal carcinoma, and asthma (Li et al., 2015; Gong et al., 2012; Guo et al., 2008).

Moreover, *GSTM1* and *GSTT1* were commonly being studied due to the high prevalence of both gene deletion in human populations compared to the other GST variants (Alshagga et al., 2011). These two genes were also suggested to show ethnic-dependent polymorphism where the frequency appeared to be dissimilar in different populations. The general consensus from numerous investigations have stated that *GSTM1* absents in approximately 50% of Caucasian and Asian populations, and in 20% of African descents (Bolt & Thier, 2006). In contrast, the prevalence of *GSTT1* deletion is substantially higher in Asians (50%) and lower in Caucasians and Africans with only 20% (Bolt & Thier, 2006).

1.2 Problem Statement

Null polymorphism of *GSTM1* and *GSTT1* present at a high frequency in most populations, particularly American, African, European, and Asian populations (Saitou & Ishida, 2015). To date, there are only few studies on *GSTM1* and *GSTT1* polymorphism conducted on Malaysian population. Malaysia is a country consists of citizens from different ethnic backgrounds; the majority of them are Malays, followed by Chinese and Indians (Department of Statistic Malaysia, 2016). As previous GST studies did not include samples from different ethnics in Malaysia, further study is needed to investigate the status of polymorphism for the two genes on the three major ethnics groups in this country. In addition, inter-individual differences on *GSTM1* and *GSTT1* null genotypes have been observed attributably to ethnicity and it was suggested that ethnicity should be considered as an important variable in the studies of GST genes (Nelson et al., 1995).

Moreover, the previous GST studies provided contradicting findings on the deletion polymorphism in Malaysia and contributed to the wide range of the null frequencies, which are 9-66% for *GSTM1* and 18-63% for *GSTT1* (Etemad et al., 2016; Eshkoor et al., 2012; Nurfatimah et al., 2011). The inconsistency could be due to the limited sample size of the control group in each study which is restricted to an average of 130 individuals only and unable to strongly determine the distribution of the genetic polymorphism in Malaysia. So, a similar study with a larger sample size of healthy individuals is significant to provide a greater statistical power in order to represent a reliable control database in Malaysia.

Genetic polymorphism of both genes in Caucasian population has become the benchmark for GST studies to determine whether the rate of gene deletion in a study population is relatively higher or lower among human populations (Garte et al., 2001). A standard DNA collection of Caucasians from European Collection of Cell Culture Human Random Control (ECACC HRC) is available as genome reference

materials in numerous studies (http:/www.hpacultures.org.uk/products/dna/hrcdna/hrcdna.jsp). However, there is little information of *GSTM1* and *GSTT1* polymorphism on the ECACC Caucasian samples. Therefore, it is relevant to investigate the status of both genes in the product samples to provide reference genotype data of *GSTM1* and *GSTT1* from Caucasian population and to precisely compare the rate of polymorphism with the findings in Malaysia.

1.3 Objectives

To investigate the distribution of *GSTM1* and *GSTT1* polymorphisms among three major ethnic groups in Malaysia and Caucasians.

Specific objectives:

1. To genotype *GSTM1* and *GSTT1* gene polymorphism in each individual in this study using multiplex polymerase chain reaction (PCR).
2. To compare the frequency of *GSTM1* and *GSTT1* gene polymorphisms among three major Malaysian ethnics and Caucasians using Chi Square test.
3. To compare the prevalence of *GSTM1* and *GSTT1* genotypes in the cohort of the study in Malaysia with Caucasians.

1.4 Hypothesis

Null genotype of *GSTM1* and *GSTT1* were hypothesized to show differences in frequency among three major ethnics (Malay, Chinese and Indian) in the general population of Malaysia. The frequencies of the null genotypes in Malaysia were expected to be different compared to Caucasians and other populations from previous studies.

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