ORIGINAL ARTICLE

Prevalence of *GSTT1* and *GSTM1* Gene Polymorphisms among Malaysian Pregnant Women

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ABSTRACT

Introduction: *GSTM1* and *GSTT1* genes polymorphisms have been widely studied whereby the absence of these genes in afflicted individuals may contribute to the risk of environment-related cancer or diseases. Thus, the present study aimed to determine the prevalence of *GSTM1* and *GSTT1* polymorphism in pregnant women, who are susceptible to clinical disease during pregnancy such as pre-eclampsia, gestational diabetes, and others. **Methods:** Blood samples were obtained on a voluntary basis. A total of 215 healthy pregnant women were recruited and their blood samples were obtained to analyse the presence of both genes, with the multiplex polymerase chain reaction (PCR) method used to simultaneously amplify the genes. **Results:** The prevalence of individuals with *GSTM1* and *GSTT1* null genotypes were 148 (69%) and 82 (38%) respectively, and 58 (27%) were having homozygous null genotype for both the genes *GSTM1* and *GSTT1* simultaneously. The studied population was compared with reported prevalence from other worldwide populations, as well as with those from other ethnic groups; Caucasian and Asians. The prevalence of homozygous null GSTM1 and GSTT1 genotype are significantly higher in the study population as compared to Caucasians and other Asians. **Conclusion:** As a conclusion, pregnant women with the genetic susceptibility were at a higher risk of clinical diseases and this data provide basis for future epidemiological studies which related to genetic variation. The limitation of this study was the lack of participation from non-Malay. Race factor is suggested to be considered in understanding the underlying role of studied genotypes.

Keywords: Prevalence of *GSTM1* and *GSTT1*, Malaysian, PCR method, susceptible group.

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INTRODUCTION

Glutathione S transferases (GSTs) are phase II metabolising and detoxification enzymes that play a significant role in cellular defence against oxidative stress and cell resistance to against drugs (1-2). The phase II enzymes are key attributes in the biological transformation of toxic compounds into a form that can be eliminated and allows the metabolic inactivation of pharmacologically active substance (1). The identification of null genotyped as the absence of an active isoform in formation of homozygous, which is contributed from the null genotypes of *GSTM1* and *GSTT1* genes (3). In humans, allelic forms of GSTs are known, often resulting in changed efficiencies of that particular enzyme. Consequently, this may result in genetically controlled reactive metabolites or a larger persistence (and thus availability) of a reactive substance in the body (4). Therefore, GSTs genes polymorphisms can modify an individual's risk towards toxicant exposure (5).

GSTM1 belongs to the GST mu (μ) cluster, which is found on chromosome 1 in region 1p13.3 and play a role in the detoxification of polycyclic aromatic hydrocarbons and other mutagens. While for the *GSTT1* gene, it is found in the chromosome 22q11.2 and

involved in the metabolism of tiny molecules present in tobacco smoke (6). The polymorphism in *GSTM1* and *GSTT1* gene loci is referred to the gene deletion which results in the absence of enzyme activity in individuals with the *GSTT1* and *GSTM1* null genotypes (7). The association of *GSTM1* and *GSTT1* gene polymorphisms with related diseases has been widely reported, which includes cancer (8-10), diabetes (11-12) renal disease (13), preeclampsia (14) and anti-tuberculosis drug-induced hepatoxicity (15). Furthermore, these works have established the prevalence of *GSTM1* and *GSTT1* polymorphisms among the worldwide population. The Asian and Caucasian populations are particularly reported to be having the highest prevalence of null of such polymorphisms compared to Europe and African populations (1,16).

The World Health Organization (WHO) has defined pregnancy as women carrying a developing embryo and fetus in their womb for nine months or so, whereby the journey is associated to an exposure to numerous health risks for the mother and developing child both. Some common health condition that pregnant women may experiences include anemia, urinary tract infection, hypertension, diabetes and other infections (17) as well as adverse pregnancy outcome such as stillbirth, miscarriage, preterm birth and abortion. Previous research associated the *GSTM1* and *GSTT1* polymorphisms with pre-eclampsia (14,18) gestational diabetes (19-20), adverse pregnancy outcome (21) and pregnancy loss (22). Pregnant women may be exposed to a variety of chemicals that have a toxic reproductive agent. The exposure to the variety of chemical among pregnant women can be explained through dietary intake, occupational exposure and air pollution. The historical incidence of Minamata Disease in Japan revealed that, pregnant women who consumed the contaminated fish only showed mild or no symptoms but the severe developmental disabilities, including cerebral palsy, mental retardation, and seizures can be seen in their infants (23). However, there are not necessarily all exposed have adverse pregnancy outcome. In the end, the metabolism is essential to detoxification mechanism.

A smoking pregnant mother has the probability of being considered as a possible threat for detrimental birth outcomes such as reduced in birth weight (< 2500 g), intrauterine growth restriction (IUGR, < 2500 g) and small gestation (≥ 37 weeks), while the *GSTM1* and *GSTT1* gene polymorphism may contribute to the individual's difference response to tobacco smoke (21). The finding suggested that the light-smoking mothers with the *GSTM1* null polymorphism have a greater low birth weight (LBW) risk (OR 1.91; 95% CI 0.43 – 8.47) than that of the *GSTM1* wild type. An absence in the *GSTT1* gene was significantly associated with the small gestation among pregnant mothers exposed to the benzene at their workplace, even below than permissible limit set by Occupational Safety and Health Administration (OSHA) (24).

The researchers had explored the role of genetic background in mediating the individual susceptibility to chemical exposure for example mercury. The embryonal system can have impact from the exposure of mothers to dietary intake of contaminated fish besides the exposure through occupational and amalgam fillings. The associated outcome from the dietary exposure were low birth weight, stillbirth, hypoplasia of the cerebellum, decreased number of nerve cells in the cerebral cortex, decreased total brain weight, abnormal neuron migration and spontaneous abortions (25). A study conducted by Lee et al. (26) on the investigation of gene–environment interactions between blood Hg and GST polymorphisms on birth weight showed that, the mothers with the *GSTT1* null genotype, the increased Hg levels in maternal blood during late pregnancy were associated with an increased risk of lower birth weight in infants.

Pregnant women with GST genes polymorphism have a higher tendency to be susceptible to adverse health effects related to environmental factor. The research in Malaysia has been focusing on evaluating GST gene polymorphisms (e.g. 18,27-28), but as far as we can tell, none has evaluated the prevalence of GST gene polymorphisms among pregnant women with environmental factors and other gene-related disease. Therefore, the objective of this study is to determine the distribution of *GSTM1* and *GSTT1* gene polymorphisms among pregnant women, a high-risk group and may susceptible to the adverse health effect in their infants. The findings of this study can contribute to the basic data on the prevalence of *GSTM1* and *GSTT1* polymorphisms and act as an early indicator to adverse health effects in the population and help formulate a better way for prevention measures, especially among susceptible groups.

MATERIALS AND METHODS

Study Design and Respondents

This cross-sectional study was carried out from December 2016 to August 2018 among pregnant women who came for routine check-ups in Maternal and Child Health Clinics based in the Petaling District, Selangor. There were seven clinics involved in this study and each of the clinic was identified for the sample size by using area probability sampling technique to recruit the respondents and samples. The total number of sample size were summed up for all seven clinics until it reached the desired sample size which is 215. The healthy pregnant women with no medical condition were enrolled in this study. Ethical approval was obtained from the UPM Ethic Committee for Research Involving Human Respondent (JKEUPM) [Ref: UPM/TNCPI/RMC/1.4.18.2(JKEUPM)] and Medical Research and Ethic Committee (MREC) of Ministry of Health

(MOH) (NMRR ID: 16-782-30590). The inclusive criteria for this study were healthy Malaysian pregnant women aged between 20 to 49 years old and had no chronic diseases. The respondents were introduced with the study's objective and signed the consent form as they agreed to voluntarily participate in this study. Blood samples were collected in lavender-cap tube (BD Vacutainer) containing heparin. The blood samples were temporarily stored in cool box while transported to the laboratory and then stored in the temperature of -20^{0}C prior to analysis.

GSTM1 and *GSTT1* genotyping

The genotyping of *GSTM1* and *GSTT1* genes were performed by multiplex PCR as described by Cheng et al. (29) and Uddin et al. (30). The DNA extraction was from the manufacturer's protocol QIAGEN QIAmp (Qiagen, Inc., Chatsworth, CA). The albumin gene was selected as an internal control. Briefly, the 10 µl PCR mixture contained 1 µl of 20 ng of template DNA, 0.5 µl of 10 µm of each *GSTM1* and *GSTT1* primers, 4.5 µl of 2X prime Taq premix and 1.5 µl sterile ultrapure water. Both forward and reverse primers used were from Matic et al. (31) and the sequence are as follow: *GSTM1* –(F: 5´-GAACTCCCTGAAAAGCTAAAGC-3´, R: 5´-GTTGGGCTCAAATATACGGTGG-3´), *GSTT1* –(F: 5´-TTCCTTACTGGTCCTCACATCTC-3´, R: 5´-TCACCGGATCATGGCCAGCA-3´), and albumin –(F: 5'- GCCCTCTGCTAACAAGTCCTAC- 3', R: 5'-GCCCTAAAAAGAAAATCGCCAATC- 3') (32). The PCR was performed at the initial denaturation at 94^{0}C for 5 min, then denaturation at 94^{0}C for 90 sec and then annealing at 62^{0}C for 1 min. Then, the samples were subjected to extension at 72^{0}C for 1 min and final annealing at 62^{0}C for 5 min before the final extension was undertaken at 72^{0}C for 10 min. This amplification process takes 35 cycles for all steps. Lastly, the PCR by-product was subjected to 3% of agarose gel electrophoresis to present the *GSTM1* and *GSTT1* bands, which are expected to be 215 bp and 459 bp, respectively.

Statistical Analysis

The statistical analysis was performed using SPSS statistical software Version 23. The descriptive analysis was used to measure the prevalence of GSTM1 and GSTT1 polymorphisms among the respondents.

RESULTS

Figure 1 show the example of PCR product for selected samples, whereby the null genotype is identified if the band of each gene is absent. Individuals in sample lanes 2, 4, and 7 had *GSTT1* null, while lines 8, 10, and 11 had *GSTM1* null. Meanwhile, lines 5 and 6 had *GSTM1* and *GSTT1* wild genotype, whereas lines 3 and 9 had both *GSTM1* and *GSTT1* null. The prevalence of *GSTM1* and GSTT1 deletion polymorphisms in this presence study were 69% and 38%, respectively and is consequently shown in Table I, whereby GST- indicates the absence of the gene while GST+ indicates its presence. The highest prevalence obtained was for *GSTM1*-/*GSTT1*+ with a value of 43%, which was then followed by *GSTM1*-/*GSTT1*-; *GSTM1*+/*GSTT1*+; *GSTM1*+/*GSTT1*-; and with the values of, 27%, 20% and 11% respectively. Table II reveals the prevalence data of GSTM1, GSTT1 and combination of *GSTM1* and *GSTT1* null genotype between this study and across worldwide populations study, explaining the varied number of prevalence across the population.

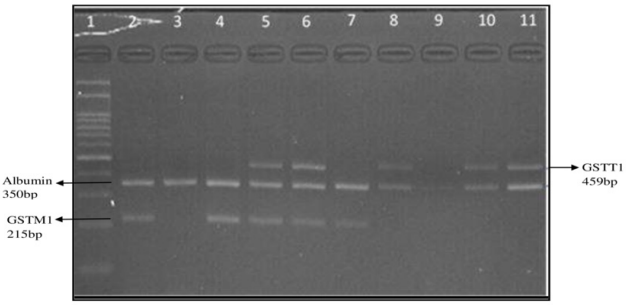

Figure 1: Example of the PCR product for selected samples. Agarose gel (3%) image of multiplex *GSTM1* and *GSTT1* PCR. Band of 350bp is Albumin, the internal control. The presence of the bands of 459bp and 215bp denotes the expression of *GSTT1* and *GSTM1* genes, respectively. Accordingly, lines 2,4 and 7 show individuals that express only *GSTM1*, lines 8,10 and 11 show individuals that express only *GSTT1* gene and lines 5 and 6 show individuals express both *GSTM1* and *GSTT1* genes while lines 3 and 9 show individuals who do express neither *GSTM1* nor *GSTT1* genes.

Table I. Prevalence of GSTs genes for single and combination polymorphism

Genotype	Prevalence (%)
GSTM1	
Null	69
Wild	31
GSTT1	
Null	38
Wild	62
GSTM1+/GSTT1+	20
GSTM1-/GSTT1+	43
GSTM1+/GSTT1-	11
GSTM1-/GSTT1-	27

GSTM1+/*GSTT1*+: both genes wild, *GSTM1*+/*GSTT1*-: *GSTM1* wild /*GSTT1* null, *GSTM1*-/*GSTT1*+: *GSTM1* null/*GSTT1* wild, *GSTM1*-/*GSTT1*-: both genes null

DISCUSSION

The homozygous deletion of *GSTM1* and *GSTT1* gene have an impact on the functionality of detoxification enzyme to eliminate the toxic compound and may deteriorate the health condition of individuals to various disease. The present study aimed to determine the *GSTM1* and *GSTT1* polymorphism among one of the susceptible groups in Malaysia, which is pregnant women. The study findings may contribute towards understanding and providing a baseline data for future research in genetic or gene-related disease studies, particularly in pregnant women.

Table II Prevalence of *GSTM1* and *GSTT1* null genotype in pregnant women population.

Country	N	GSTM1 Null (%)	GSTT1 Null (%)	Both null (%)	Reference
Malaysia	**215**	**69**	**38**	**27**	**Present study**
Japan	160	45.6	49.4	22.5	22
China	293	59.4	50.2	29.7	14
China	265	32.8	29.4	NA	20
Taiwan, China	106	47.2	52.8	NA	25
Korea	782	56.5	52.6	NA	39
Korea	417	60	53	NA	27
Romania	405	5.9	14.3	NA	34
Iran	200	50.5	26	15	38
India	180	29	3	1	35
Mexico	233	44.2	10.3	2.57	18
France	348	49	17	NA	36
Lithuania	460	47	15.4	NA	37

This study conducted the prevalence of *GSTM1* and *GSTT1* gene polymorphisms among pregnant women in Selangor state, Malaysia. The data obtained was compared with the study among pregnant women from worldwide population. The baseline frequencies of null genotypes for *GSTM1* and *GSTT1* in different ethnic groups varies significantly. In the present study, 69% individuals with *GSTM1* homozygous null genotype in Malaysian pregnant women population which is comparable with data reported from Korean pregnant women population (26) (Table 2) and is consider high compared to the pregnant women in Romania, (5.9%) China (32.8%) and India (29 %), where the frequency is very low (20, 33-34) respectively. Other studies reported the prevalence of *GSTM1* null between 44.9% to 59.4% were from Mexico, Lithuania, France, Iran, China, Korea and Japan (14,18, 22,35-38).

Meanwhile, in the current research, the prevalence of *GSTT1* null was 38% which was higher than other studies from Romania, Iran, India, Mexico, France and Lithuania, where the prevalence was between 3% to 19.1% (18,33-36). The prevalence of *GSTM1* homozygous null genotype is significantly higher in Asian (range: 29% – 60%) as compared to Caucasian (range: 5.9%– 49%) while the prevalence for *GSTT1* homozygous null genotype was higher in Asian (3% - 53%) as compared to Caucasian (range: 10%-17%). The prevalence of both null genotypes is higher in Asian (range: 22.5%-29.7%) as compared to Caucasian (2.57%). There is limited data available for the combined homozygous null genotypes of *GSTM1* and GSTT1.

The prevalence of *GSTM1* null genotype among pregnant women in Malaysian population in this study was higher compared to those of other countries. This suggest that the Malaysian population in this study is distinct and are not descendant from the European population. It also can be concluded that a higher prevalence of *GSTM1* null was found among the Malaysian population than those of other countries. This phenomenon will contribute to a new incidence of xenobiotic exposure (18) and draw a special attention towards such distinction due to the influence of few factors. The factors are; "different of evolutionary history of each population, selection based on different lifestyle habits, different exposure to toxins, and differential susceptibility to certain diseases" (1 p. 1228).

It was observed that the prevalence of *GSTM1* null found in this study and other Asian population such as Japanese, Chinese, Indian, Iranian and Korean were higher in frequency compared to frequency distribution in *GSTT1* null. However, the distribution of *GSTT1* null was varied across all countries and continents. This variation may be due to the geographical location of the study and the inter-ethnicity of the population studied. The deleterious of *GSTM1* gene will lead to the higher chance of loss of functional activities. Therefore, GST gene variation might have a pivotal role in the relationship for a certain gene-environment association study, particularly an exposure to a high-risk group such as pregnant women. Hence, these findings have underlined the need for government policies and guidelines for minimising the exposure of such harmful chemical to a high-risk group, especially pregnant women with the genetic susceptibility.

Pregnant women with the genetic susceptibility were at a higher risk of clinical diseases and environmental-related disease and this data provide an early detection for health outcomes in pregnant women and provide basis for future studies which related to genetic variation. There is a limitation in the current study that should be taken into consideration to further improve its quality in the future. This study did not reveal the prevalence of *GSTM1* and *GSTT1* polymorphisms for various ethnicities in Malaysia. Thus, the GSTs null polymorphisms could not be determined between the various ethnics available in Malaysia. This improvement could be considered for future research plan to obtain a more comprehensive understanding of *GSTM1* and *GSTT1* polymorphisms in Malaysia.

CONCLUSION

The study population has a high prevalence of *GSTM1* and *GSTT1* null genotype, which is quite a similar rate as reported by other studies in several Asian countries. The results could be used as baseline data to implement health monitoring among pregnant women in Malaysia related to the genetic polymorphisms of *GSTM1* and *GSTT1* and can be used to find the relation of gene polymorphisms with various type of diseases as well as effects of environmental exposure. Furthermore, this

data is beneficial for future research in planning the studies related to the polymorphisms of *GSTM1* and *GSTT1* genes among pregnant women and in comparing the trend results from different ethnics in Malaysia.

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