Siti Aishah Zainal[1], Mohd Aminudin Mustapha[2], Ahmad Aizat Abdul Aziz[3], Siti Nurfatimah Mohd Shahpudin[4]*

[1]School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

[2]Centre for Pre-University Studies, Universiti Malaysia Sarawak (UNIMAS), 94300 Kota Samarahan, Sarawak, Malaysia.

[3]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.

[4]Department of Biomedical Sciences, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Sains@Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia.

*Corresponding author
Siti Nurfatimah Mohd Shahpudin
Sitinurfatimah.ms@usm.my

Association of High-Risk Polymorphisms in Xenobiotic Metabolising Genes (GSTM1, GSTT1 and GSTP1) and Their Association with Colorectal Cancer Susceptibility

Abstract – Colorectal cancer (CRC) is an increasing health concern in Malaysia, ranking as the second most common cancer overall—first among men and second among women. Many cases are diagnosed at advanced stages, when treatment options are limited and survival rates are low. Identifying high-risk individuals early is therefore essential. Genetic variations in xenobiotic-metabolising enzyme (XME) genes, which regulate the detoxification of carcinogens, may influence individual susceptibility to CRC. In this case–control study, three XME polymorphisms including GSTM1 and GSTT1 gene deletions and the GSTP1 Ile105Val (A313G) single nucleotide polymorphism—were examined in 255 patients with histologically confirmed CRC and 311 age- and sex-matched healthy controls. Genotyping was performed using multiplex PCR and PCR–RFLP. Logistic regression adjusted for age, sex, and ethnicity showed that the GSTP1 Val/Val and GSTT1 null genotypes were linked to higher CRC risk. Combined genotypes, including GSTM1 null/GSTT1 null and triple-null variants, conferred the greatest susceptibility. These findings support the use of XME genetic markers in targeted screening and precision prevention, offering a pathway to reduce late-stage diagnoses and improve CRC outcomes in Malaysia.

Keywords *– **Colorectal cancer (CRC); Glutathione S-transferases (GSTs); Cytochrome (CYP); Single nucleotide polymorphisms (SNPs); Genetic predisposition.***

1 INTRODUCTION

The increasing incidence of colorectal cancer (CRC) in developing countries, including Malaysia, underscores the urgency of identifying genetic biomarkers for early detection and prevention [1]. CRC is a multifactorial disease resulting from complex interactions among environmental exposures, lifestyle habits and genetic predispositions. Polymorphisms in xenobiotic-metabolising enzyme (XME) genes may influence carcinogen activation and detoxification, thus modulating individual susceptibility to CRC. Downregulation of multiple XMEs in tumour tissue suggests a cumulative effect on carcinogen metabolism [2]. This study hypothesises that genetic variations in XME genes may affect individual responses to carcinogenic compounds and thereby influence CRC susceptibility in the Malaysian population. Carcinogens detoxified by GSTs include polycyclic aromatic hydrocarbon present in the diet and tobacco smoke. The three forms of GSTs (GSTMI, GSTTI and GSTPI) have been analyzed in different population groups for polymorphic variants and to elucidate their role in carcinogenesis. But no reports are available from Malaysia. So, a case control study was undertaken to determine the polymorphic allele frequencies of GSTM1, GSTT1 and GSTP1 (Ile105Val) in Malaysian healthy controls and CRC patients and to evaluate the influential role of these variant genotypes in CRC susceptibility risk

2 MATERIALS & METHODS

2.1 Patient Recruitment

This case–control study included a total of 566 individuals, consisting of 255 patients with histologically confirmed CRC and 311 healthy controls matched by age and sex. The mean age was 44.5 years with a range between 38 to 72 years. Control groups consisted of healthy individuals with no history from HNPCC, FAP and other cancers involved in 72 men and 56 women with a mean age of 58.4 years, ranging from 30 to 68 years. Informed consent was obtained from all patients and controls. Personal details, dietary habits and other data were collected through an interview based on standardized questionnaire.

2.2 Sample Collection

After getting written informed consent, 3ml peripheral blood was collected from all study subjects in EDTA vacutainer tubes. Immediately after collection, whole blood was stored at -20°C until used. Genomic DNA was extracted from 200µl whole blood using DNA extraction kit (QIAGEN, Germany).

2.3 Genotyping

2.3.1 *GSTPI* polymorphism

Ile105Val polymorphism was analyzed by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) technique with composition PCR mastermix using approximately 100ng DNA, primer (0.2nM), 2.0mM MgCl2, 10X PCR buffer, 10mM dNTp and 5U Taq DNA polymerase (Applied Biosystem) with total volume of 25µl. The annealing temperature was 56°C and 30 PCR cycles were carried out. The sequences of the primers are shown in Table 1. The PCR product (5 µl) was digested with 1 U BsmAI and 1X Buffer in total volume of 10 µl at 37°C for 1 hour incubation. The PCR fragment were separated on 2% agarose gel (VIVANTIS) and visualized under ultraviolet trasilluminator with Sybr Green. The genotypes were categorized according to the band sizes with the PCR product at 433bp. For individuals with *GSTPI* homozygous wlldtype (Ile/Ile/AA) genotype are detected with bands of 329bp and 104bp, the heterozygous variant (Ile/Val/AG) genotype are visualized with 3 bands of 329bp,225bp and 104bp and for homozygous variant (Val/Val/GG) genotype are detected with 2 bands at 225bp and 104bp (Table 1).

2.3.2 *GSTMI* and *GSTTI* polymorphisms

A multiplex polymerase chain reaction method was used to detect the presence or absence of the *GSTMI* and *GSTTI* genes in genomic DNA samples and *β-globin* gene was included as housekeeping gene. The sequences of the primers are shown in Table 1. The PCR composition for *GSTMI*, *GSTTI* and *β-globin* were at final volume of 25 µl and the PCR product were separated on 3% agarose gel and visualized under ultra violet trasilluminator with Sybr Green. The best annealing temperature for all 3 primers was 56.4°C and 35 cycles were carried out. The absence or presence of *GSTMI* and *GSTTI* specific PCR product indicated the corresponding null genotype whereas the β-globin specific fragment confirmed the proper functioning of the reaction.

3.0 Result and Discussion

Significant associations were found between the *GSTP1* Val/Val and *GSTT1* null genotypes and an increased risk of CRC. Individuals with combined genotypes, such as *GSTT1* null/*GSTM1* null and *GSTT1* null/*GSTP1* Ile/Ile or Ile/Val, showed even higher susceptibility. These results are supported by earlier studies. For example, the *GSTT1* null genotype was linked to a higher CRC risk (OR = 1.66; 95% CI: 1.20–2.31), and the *GSTM1* null genotype also contributed to increased risk (OR = 1.57; 95% CI: 1.13–2.18) [3]. The risk was further elevated with the combined *GSTM1* null/*GSTT1* null genotype (OR = 1.95; 95% CI: 1.33–2.85) [3]. The highest risk was observed in individuals with both *GSTM1* null and *GSTP1* or *GSTT1* variants (OR = 3.00; 95% CI: 1.20–8.10) [4]. Those with the triple-null genotype (*GSTM1* null/*GSTT1* null/*GSTP1* Ile/Ile) had the highest risk of CRC (adjusted OR > 2.5), suggesting a strong combined effect on detoxification ability.

The *GSTM1* null genotype was most common among Malays (66%) [5]. These results underline the role of genetic differences between ethnic groups and how lifestyle factors such as diet, smoking and working exposure may influence CRC risk. Gene-environment interactions in Southeast Asians differ from those in Western populations [6,7,8], highlighting the need for local studies on genetic biomarkers. In addition, the changes in XME genes may interfere with the body's ability to neutralise carcinogens, leading to the accumulation of DNA damage and the initiation of carcinogenesis.

4.0 Conclusion

This study shows that genetic differences in xenobiotic metabolism genes, alone or combined, play an important role in colorectal cancer risk. Identifying these high-risk genes could support the development of genetic tests to better identify individuals at risk and improve targeted screening. This is consistent with the shift toward personalised medicine and precision cancer care. Proteomics, metabolomics and transcriptomics combined in future studies may improve risk prediction and biomarker discovery.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Ministry of Higher Education (MOHE) under the Fundamental Research Grant Scheme (FRGS) FRGS/1/2022/STG01/USM/02/1

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Table 1. Association of 2-way combination genotypes of *GSTM1*, *GSTT1* and *GST1*

Combination Genotypes	Cases n=255 (%)	Controls n=311 (%)	OR (95% CI)	P value	[a]OR (95% CI)	P value
GSTM1* and *GSTT1						
Both present	186 (72.9)	267 (85.9)	1.000 (Ref)		1.000 (Ref)	
Both Null	69 (27.1)	44 (14.1)	2.251(1.476-3.433)	**<0.001***	2.062 (1.262-3.370)	**0.004***
GSTM1* and *GSTP1						
M1(+/+) & P1(Ile/Ile)	53 (20.8)	74 (23.8)	1.000 (Ref)	-	1.000 (Ref)	-
M1(+/+) & P1(Ile/Val)	42 (16.5)	56 (18.0)	1.047 (0.614-1.785)	0.865	1.122 (0.603-2.090)	0.716
M1(+/+) & P1 (Val/Val)	11 (4.3)	3 (1.0)	5.119 (1.362-19.249)	**0.016***	9.504 (1.890-47.792)	**0.006***
M1 (-/-) & P1(Ile/Ile)	74 (29.0)	111 (35.7)	0.931 (0.588-1.473)	0.760	1.044 (0.614-1.775)	0.873
M1 (-/-) & P1 (Ile/Val)	62 (24.3)	61 (19.6)	1.419 (0.061-2.338)	0.169	1.453 (0.817-2.582)	0.294
M1 (-/-) & P1 (Val/Val)	13 (5.1)	6 (1.9)	3.025 (1.080-8.471)	**0.035***	2.715 (0.836-8.817)	0.096
GSTT1* and *GSTP1						
T1(+/+) & P1(Ile/Ile)	54 (21.2)	133 (42.8)	1.000 (Ref)	-	1.000 (Ref)	-
T1(+/+) & P1 (Ile/Val)	47 (18.4)	83 (26.7)	1.395 (0.865-2.248)	0.172	1.275 (0.736-2.208)	0.386
T1(+/+) & P1 (Val/Val)	11 (4.3)	7 (2.3)	3.870 (1.425-10.510)	**0.008***	5.539 (1.634-18.781)	**0.006***
T1(-/-) & P1(Ile/Ile)	73 (28.6)	51 (16.4)	3.525 (2.187-5.683)	**<0.001***	3.114 (1.799-5.391)	**<0.001***
T1(-/-) & P1 (Ile/Val)	57 (22.4)	35 (11.2)	4.011 (2.369-6.791)	**<0.001***	3.878 (1.789-5.391)	**<0.001***
T1(-/-) & P1 (Val/Val)	13 (5.1)	2 (0.6)	16.009 (3.495-73.339)	**<0.001***	9.987 (1.975-50.498)	**0.005***

*Statistically significant, $p<0.05$
[a]Adjusted for gender, age and ethnicity