

**GENETIC POLYMORPHISM OF DRUG METABOLIZING ENZYMES AND
ESTROGEN RECEPTOR IN PHARMACOGENETICS OF TAMOXIFEN:
IMPLICATION FOR OPTIMIZATION OF BREAST CANCER TREATMENT**

**INSTITUT PENGURUSAN PENYELIDIKAN (RMI)
UNIVERSITI TEKNOLOGI MARA
40450 SHAH ALAM, SELANGOR
MALAYSIA**

**BY :
TEH LAY KEK
MOHD ZAKI SALLEH**

DISEMBER 2010

PROJECT TEAM MEMBERS

ASSOCIATE PROF. DR TEH LAY KEK

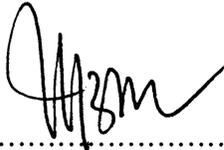
Project Leader



.....
Signature

PROF. DR. MOHD ZAKI SALLEH

Project Member



.....
Signature

Abstract

Introduction: Tamoxifen has been used as a hormonal therapy in breast cancer patients who are positive for estrogen receptor. The drug is metabolized by Cytochrome P450 2D6 (*CYP2D6*) into several metabolite. Variation in *CYP2D6* activity has important therapeutic consequences and can play a significant role in the development of adverse events or therapeutic failure in susceptible individuals. Beside, variation of drug transporter such as *MDR1* may alter the accumulation of the drug and cause toxicity in patients. Furthermore, the different expression of receptor- α and estrogen receptor- β may be associated with different therapy outcome.

Materials and methods: In subject recruitment, patient samples were collected from HUKM, Hospital Selayang and HTAF. Patients who have received tamoxifen for treatment of breast cancer were recruited according to exclusion and inclusion criteria. Genotyping method for *CYP2D6* and *MDR1* were developed using multiplex allele specific PCR (ASPCR) approach. DHPLC method was developed to detect existing and new alleles in *CYP2D6* and estrogen receptors. The expression of estrogen receptor- α and estrogen receptor- β from samples would be quantitated using Real-time PCR.

Result: The most common variants detected is *CYP2D6*10* with 50% of heterozygous *CYP2D6*1/*10* and *CYP2D6*5* with 7.8% was detected high in breast cancer patients. Furthermore *CYP2D6*1/*4* and *CYP2D6*1/*4* was detected but at low frequencies.

Table of Contents

Abstract	I
Acknowledgement	III
Table of Contents	IV
List of Table	XI
List of Figure	XII
List of Plates	XV
Chapter 1 Literature Review and Introduction	
1.1 Breast Cancer	1
1.2 Incidence of Breast Cancer in Malaysia	2
1.3 Hormonal Treatment for Breast Cancer	3
1.3.1 Tamoxifen	3
1.3.2 Metabolism of Tamoxifen	5
1.3.3 Problems in Tamoxifen Treatment	6
1.4 Polymorphism of <i>CYP2D6</i>	9
1.5 Role of Drug Transporter In Breast cancer Treatment	14
1.5.1 MDR1 Gene, P-gp and Tamoxifen	15
1.6 Estrogen Receptor	16
1.7 Statement of Problems	20
1.8 Objectives	21

Chapter 2 Study Design and Methodology

2.1	Study Design	22
2.1.1	Overall Study Design	23
2.1.2	Method Development and Analysis	24
2.2	Scopes of the Research	25
2.2.1	Subjects	25
2.2.2	Inclusion Criteria	25
2.2.3	Exclusion Criteria	25

Chapter 3 Development and Validation of Allele Specific PCR and dHPLC for *CYP2D6* Variants Detection

3.1	Introduction	26
3.2	Materials	27
3.3	Methodology for Allele Specific PCR	
3.3.1	Selection of Primers	29
3.3.2	Protocol for Detection of <i>CYP2D6</i> variants	
	3.3.2.1 First PCR Master Mix Preparation	32
	3.3.2.2 Allele Specific PCR Master Mix Preparation	
3.4	Result	
3.4.1	Integrity of the DNA	34
3.4.2	Amplification of Fragment A and B of First PCR	35
3.4.3	Amplification of <i>CYP2D6</i> Variants	36
3.5	Validation of <i>CYP2D6</i> Genotyping	37
3.6	Protocol for Detection of <i>CYP2D6</i> *2 <i>XN</i> and <i>CYP2D6</i> *5	