

UNIVERSITI TEKNOLOGI MARA

**DETECTION OF TPMT*3C GENE
POLYMORPHISM BY POLYMERASE CHAIN
REACTION (PCR) IN RELATED TO
AZATHIOPRINE MEDICATION**

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ABSTRACT

Azathioprine is an immunosuppressive drug used to treat inflammatory bowel diseases, rheumatoid arthritis and to prevent rejection in organ transplantation. It is metabolized mainly into inactive 6-methylmercaptopurine (6-MMP) via thiopurine S-methyltransferase (TPMT) enzyme. The deficiency of TPMT enzyme due to genetic polymorphism has a higher risk of developing severe, life-threatening myelotoxicity when given standard doses of azathioprine. The Nested PCR method was used to detect the SNPs in TPMT*3C (rs1142345). It is one of the major allele that causes the activity of TPMT enzyme to be low. The study focused on the optimization of the Nested PCR where the parameters were adjusted to obtain the optimized condition. The parameters altered in this study include the concentration of DNA template, annealing temperature and the volume of primers. The amplified PCR product was evaluated by gel electrophoresis and observed under UV light. In conclusion, the optimization of Nested PCR method was not yet achieved hence the mutant and wild type cannot be differentiated. The other parameters including the concentration of $MgCl_2$, the number of cycles in PCR execution and concentration of enzyme should be adjusted as well.

CHAPTER ONE

INTRODUCTION

1.1 Overview

Human generally contains different kind of genes which encodes the proteins needed by the body. The study of pharmacogenomics itself refers to the effects of drugs on genetic variation. Drugs basically need specific condition or in the presence of certain enzymes in order to effectively undergo absorption, distribution, metabolism and elimination inside the body. Complications may happen if one of these pharmacokinetics orders cannot be done accordingly. Due to variation in human's genotype, goal of pharmacogenomics study is to achieve maximum efficacy in optimizing the drugs therapy with minimal adverse effects as possible.

Thiopurine S-methyltransferase (TPMT) is an enzyme responsible for the S-methylation of thiopurine drugs such as azathioprine, 6-mercaptopurine and 6-thioguanine. A TPMT gene that encodes for the TPMT enzyme is located on the chromosome 6p22.3. This gene is a primary concern in terms of genetic polymorphism which will affect the level of enzyme produced in an individual. The presence of TPMT enzyme is crucial as it will inactivate the thiopurine drugs.