UNIVERSITI TEKNOLOGI MARA

GENOTYPING OF *CYP2C9* USING DNA FROM HAIR ROOTS, SALIVA, FINGERNAILS, BUCCAL CELL SWABS AND BLOOD

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ABSTRACT

Background: Blood is an excellent source of genomic DNA. However, blood sampling is invasive, painful and requires complicated handling and transport. Therefore, we intend to obtain DNA from other sources such as hairs, saliva, fingernails and buccal cell as an alternative to blood. The samplings of these non-blood specimens are painless, non-invasive, convenient and accessible as compared to blood as a source of DNA for genotyping test.

Aim: The aim of the study was to develop a simple DNA extraction method from sources other than blood. These DNA could thus be used for genotyping test of the polymorphic CYP enzymes whereby *CYP2C9*3* was used as prototype.

Method: Hairs, saliva, fingernails, buccal cell swabs and blood were taken from 5 healthy unrelated volunteers. They are students of Faculty of Pharmacy, Universiti Teknologi MARA. The samples were subjected to DNA extraction. DNA were extracted from hair roots, saliva, fingernails, buccal cell swabs by using 2 different extraction methods which were one step DNA extraction method with and without Chelex. The blood samples were extracted by using Quick Gene DNA whole blood kit S (DB-S). The extracted DNA was used for subsequent PCR based genotyping of *CYP2C9*3*.

Results: DNA was successfully extracted from hair roots, saliva, fingernails and buccal cell swabs by using both extraction methods. However, the amount of DNA extracted was not as high yield as compared to DNA extracted from blood. The DNA obtained from these different sources were able to be amplified by PCR and thus useful for genotyping of *CYP2C9*3* in comparison to DNA extracted from blood.

Conclusion: The DNA extracted from hair roots, saliva, fingernails and buccal cell swabs were useful sources of genomic DNA and thus could be used in genotyping of *CYP2C9*3*.

CHAPTER 1

INTRODUCTION

The cytochrome P450 (CYP) enzyme is one of the most important oxidative enzyme systems involved in the metabolism of many clinically used drugs. Some of the CYP enzymes are genetically polymorphic. Genetic polymorphism is a difference in DNA sequence among individuals, groups, or populations. Genetic polymorphism has been suggested to be responsible for interindividual variations in the pharmacokinetics of the drugs and the efficacy and frequency of adverse events. One of these polymorphic enzymes is CYP2C9. CYP2C9 involved in metabolism of varieties of drugs such as warfarin, phenytoin, losartan, tolbutamide and NSAIDs (Lee *et al*, 2002). There are two different methods to detect genetic polymorphism which are phenotyping or genotyping. Genotyping of this polymorphic enzyme may be useful to personalized medicine for genotypes can be used to predict phenotype and efficacy and toxicity of drugs before administration. Therefore, it is very important to identify the genotypes of this enzyme for the use in modern medicine in achieving genetically targeted drug use.

Blood is an excellent source of genomic DNA. Therefore, genomic DNA extracted from blood is generally used for genotyping. However, blood sampling is invasive and painful (Gan *et al*, 2003; Tanigawara *et al*, 2001; Ng *et al*, 2004) .The sampling procedure may be inconvenient for paediatric populations and reluctant patients (Gan *et al,* 2003). Furthermore, blood samples are complicated by handling and transport (Tanigawara *et*