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Method optimisation for CYP450, 2C19*17(3402) SNP recognition using PCR

M. Abiarghamsari[1], M. Sistanizad[1], F. H. Shirazi[2,*]

[1]Department of Clinical Pharmacy, School of pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Pharmaceutical Sciences Research Center, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background and Aims: Genetic variation, most of which are single nucleotide polymorphism (SNPs), causes inter-patient differences in drug response. The cytochromes P450 (CYP450) hold a central position in human drug metabolism. Heterozygosity or homozygosity for the increased function of *17allele is associated with the increased rate of CYP2C19 activity and ultra-rapid metabolizer phenotype in human being. This genetic variability can alter response to many medications like Clopidogrel.
Methods: This study was designed to obtain a PCR method for the detection of CYP2C19*17 gene polymorphism. DNA from Iranian population blood samples were extracted with QIAGEN kit. Different concentrations of MgCl, dNTP, Primer, Taq DNA Polymerase and DNA were used to optimize PCR condition. PCR machine was run for 40 cycles and products were analyzed by gel imagining software after gel electrophoresis.
Results: During the study 20 samples with different concentrations of material were investigated. MgCl increases band determination in a direct relation up to concentration of 3 mM. dNTP increases band determination in a direct relation up to concentration of 0.12 mM. Taq DNA Polymerase increases band determination in a direct relation up to 1.25U. Primer increases band determination in a direct relation up to concentration 400nM.
Conclusions: The best experimental condition to detect the *17 allele of CYP450C19 SNP in the human samples is as follow: PCR Buffer 2.5, $MgCl_2$, 3 mM, dNTP 0.12 mM, Taq DNA Polymerase 1.25U, DNA 2.8 ng/µl, Primer 400 nM. The validity and applicability of this method is acceptable for laboratory diagnosis and pharmacogenetic studies in patients.

Keywords: Polymorphism; CYP4502C19; PCR