DETECTION OF DRUG RESISTANCE MUTATIONS IN HUMAN IMMUNODEFICIENCY VIRUS BY MUTAGENICALLY SEPARATED POLYMERASE CHAIN REACTION

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ABSTRACT

Gene sequencing is a proven method of choice in detection of point mutations associated with Human Immunodeficiency Virus (HIV) drug resistance. The method is however very expensive and prohibitive in resource poor settings. This standard nucleotide sequencing method costs $200-$500 and involves the use of expensive equipment. There is need therefore to develop a cheaper method that is fast in detection of point mutations that are responsible for HIV-1 drug resistance.

The objective of the study was to evaluate the use of mutagenically separated PCR in detection of drug resistance mutations in HIV patients. The procedure involved the use of sets of primers (molecular markers) that are able to differentiate between wild type and drug resistance strain of virus. A total of 74 samples were collected from Mbagathi and Maragua District hospitals. Samples were collected from HIV patients who have been on anti-retroviral therapy for at least six months.

The assay involved single nucleotide polymorphism detection Polymerase Chain Reaction that consisted of one-step Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) reaction and two subsequent rounds of competitive PCR. From the gel electrophoresis experiments, bands of different base pairs were obtained. However, none of the bands obtained were of the expected 190 bp for the mutant strains and 210 bp for the wild type strains. For the 103 region with the 103 primers, amplicons of different band sizes were amplified. Some amplicons had single bands and others had more than one band all of different base pairs ranging from 100bp to 300bp. Samples amplified with 181 primers for
the 181 region had double bands of 90bp and 120bp. Hence further work should be done on the samples to determine whether the mutations were as a result of mutations or not.