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PROBE BASED MELTING CURVE ANALYSIS IN ANTI-HBV DRUG RESISTANCE GENE MUTATION DETECTION

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Chronic hepatitis B (CHB) caused by Hepatitis B virus infection is a global public health issue. Individualized treatment of CHB becomes increasingly important and a best individualized treatment plan cannot be established without the information of HBV genetic diagnosis. Nowadays, HBV DNA quantification by real-time PCR is widely used in clinical diagnosis. However, there is an urgent need to establish some simple and efficient clinical detection methods for HBV drug resistance gene mutations detection. To address these issues, we proposed a study on the establishment of convenient and efficient methods for HBV drug resistance gene mutations detection. We described a real-time PCR-based assay using melting curve analysis that could accurately detect 24 HBV nucleotide mutations at 10 amino acid positions in the reverse transcriptase region of the HBV polymerase gene. The two-reaction assay had a limit of detection of five copies per reaction and could detect 5% rtM204V in the presence of the wild-type when the overall concentration was 104 copies/μL. The assay could be finished within three h and the material cost for each sample was less than 10 USD. Clinical study using three groups of samples involving both nucleotide analogs-treated and untreated patients showed that 99.3% (840/846) samples and 99.9% (8454/8460) amino acids were concordant with PCR sequencing. The six minor mutation containing samples undetected by PCR sequencing were confirmed by co-amplification at lower denaturation temperature-PCR sequencing. In the treated patients, 48.6% (103/212) were mutant comprising lamivudine-monoresistance, adefovir-monoresistance, entecavir resistance and lamivudine+adefovir resistance, respectively. Among the untreated patients, Chinese group had more mutation containing samples than did the Pakistani group (3.3% vs 0.56%). Because of its accuracy, rapidness, wide coverage and cost-effectiveness, the real-time PCR assay could be a robust tool for anti HBV drug resistance mutations detection in resources-limiting countries.

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