



The advantage of the RealTime PCR method to sequencing in detection of mutations in the *k-ras* gene

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Abstract

Introduction & Aim: Colorectal cancer is one of the main causes of cancer related death worldwide. CRC arises from mutations in different genes. One of the most important mutations in this type of cancer is in the *k-ras* (Kirstein rat sarcoma) gene. The most important mutations in KRAS gene occur in 12th and 13th codon of the second exon. Mutations that occur in this area, are very important for predicting the response of patients to anti-EGFR therapies like ctuximab and panitumumab. In this study we aimed to study these mutations by two different methods.

Methods: In this study 10 samples from patients affected with Colorectal Cancer were taken from Imam Khomeini Hospital and Cancer Institute of Iran. DNA of these samples was extracted and thereafter PCR was performed on the second exon of *k-ras* gene. Then the PCR products were sent for sequencing analysis. Thereafter primers and probes designed by Hum Diagnostics kit for detecting the mutations in these codons by real time PCR.

Results: The results of the sequencing of patients indicated that one patient had (GGT> GTT) mutation in 12th codon and 2 patients had (GGC> GAC) mutation in 13th codon. Real Time PCR with the ARMS primers and TaqMan probe was performed for detecting GGT> GTT, GGC> GAC and GGT> AGT mutations. As a result, all detected mutations by sequencing were confirmed by Real time PCR. Additionally in two patients who showed no mutation in 12th and 13th codon by sequencing, displayed (GGT> GTT) mutation in 12th codon and (GGC> GAC) mutation in 13th codon by Hum Diagnostics Real-Time PCR kit.

Conclusion: The study also showed that sensitivity of Real Time PCR method for studying mutations in *k-ras* gene is higher than sequencing and it could detect mutations which were undetectable in PCR-Sequencing. As the sensitivity of detecting



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k-ras mutations is very important for predicting response to anti-EGFR antibodies, it should be helpful to use sensitive and fast methods such as real-Time PCR for detecting k-ras mutations.

Keywords: colorectal cancer, KRAS, PCR, RealTime PCR, cetuximab