Prognostic evaluation of *KRAS* gene mutation in colorectal cancer patients.

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Abstract

In Iraqi patients with Colorectal Cancer, the present investigation included 20 control group and 40 patients with Colorectal Cancer with ages between (26–82 years) for the identification of KRAS gene by symmetric PCR technique (RT-PCR). The risk of mutant KRAS genotype in association with colorectal cancer was assessed in terms of Odds ratio which was 2.0 (95 % confidence interval of 0.61 - 6.60). This implies that persons harboring mutant KRAS genotypes are at two-fold risk of developing colorectal carcinoma than the general population.

Keywords: Colorectal Cancer, KRAS gene.

Introduction

Colorectal cancer is the most common malignant tumor of the lower gastrointestinal tract (colon and rectum) of epithelial origin, perhaps only cancer that starts as a benign adenomatous polyp, which can last a few years to become cancer. Colorectal carcinoma considered the fourth most common malignancy affecting the gastrointestinal tract (1). The RAS gene family is among the most studied and best characterized of the known cancer-related genes. Of the three human ras isoforms, KRAS is the most frequently altered gene, with mutations occurring in 17%–25% of all cancers. Particularly, approximately 30%–40% of colon cancers carry a KRAS mutation. KRAS mutations in colon cancers have been associated with a poorer survival and increased tumor aggressiveness. Additionally, KRAS mutations in colorectal cancer lead to resistance to select treatment strategies. The detection of KRAS mutations has been associated with decreased response rates to select chemotherapeutic agents. Therefore, KRAS mutational status is a critical factor when considering the use of targeted therapies. The association of KRAS gene mutation and response to therapy was first reported in patients with metastatic colorectal cancer,

who were treated with anti-epidermal growth factor receptor (EGFR) agents. Lievre et al. were the first to report the link between the KRAS gene mutation and decreased response to anti-EGFR agent (2).The KRAS oncogene is mutated in approximately 35%-45% of colorectal cancers, and KRAS mutational status testing has been highlighted in recent years. The most frequent mutations in this gene, point substitutions in codons 12 and 13, were validated as negative predictors of response to anti-epidermal growth factor receptor antibodies. Therefore, determining the KRAS mutational status of tumor samples has become an essential tool in managing patients with colorectal cancers (2).

Material and Methods

Study samples

In this study, the patient group consisted of selected 20 control and 40 patients (male n=25 and female=15) with age ranged (26–82years) diagnosed with Colorectal Cancer in diseases teaching hospital during the period from March 2018 to the end of May 2018.

DNA extraction

Whole blood samples (5 mL) were drawn via vein puncture into tubes containing EDTA and samples were stored at −20 °C until DNA extraction. Genomic DNA was extracted from whole blood using The Quick-gDNA™ Blood MiniPrep/Zymo/U.S.A.

KRAS gene by symmetric PCR technique (RT-PCR).

The following primers of genotyping positive strand for KRAS gene F-5'-AGG CCT GCT GAAAA TGA CTG -3' R-5'-TTG GAT CAT ATT CGTCCA CAA-3' and Cy5 (Probe 1) 5'-CTT GCC TAC GCC ACC AGC TCC AACT-BHQ2-3',while genotyping negative strand for KRAS gene F 5-'AGG CCT GCT GAAAA TGA CTG-3 'R-5'-TTG GATCAT ATT CGTCCA CAA-3'andROX(Probe2) 5'-AGT TGG AGC TGG TGG CGT AGG CAAG -BHQ2 - 3'.The mixture for the PCR reaction contained 10 µL of tGoTaq Probe qPCR Master Mix, 0.2 µLProbe,0.4µL

(Forward and Reverse primer),4.0µLNuclease-free water,and5µLDNA Sample Volume. PCR conditions for these amplifications were: 5 minutes at 95˚C, denaturation 15 sec at 95˚C, alignment Annealing/Extension10sec at 55˚C, Detection(Scan) 15 sec at 72˚C and Melting curve of 15 sec at 55 -95°C.

Results and Discussion

The following genotypes related to KRAS, GC (wild genotype) and AC, AT and GT (mutant genotypes) were studied and the results are shown in table 1. The rate of the wild genotype (GC) was 24 (60.0 %) and 15 (75.0 %), in study and control groups, respectively. The mutant genotype (AC) was more frequent in study group than in control group, 8 (20.0 %) versus 3 (15.0 %), respectively; however the difference did not reach statistical significance ($P = 0.724$).

The mutant genotype (AT) was equally frequent in study and control groups, 2 (5.0 %) versus 1 (5.0 %), respectively ($P = 0.724$). The mutant genotype (GT) was more frequent in study group than in control group, 6 (15.0 %) versus 1 (5.0 %), respectively; however the difference did not reach statistical significance ($P = 0.394$), as shown in table 3.5. Overall, the frequency of mutant genotypes was more in study group than in control group, 40 % versus 25 %, respectively, but the difference was not significant in statistical terms (P = 0.251), as shown in table 2.

The risk of mutant KRAS genotype in association with colorectal cancer was assessed in terms of Odds ratio which was 2.0 (95 % confidence interval of 0.61 - 6.60). This implies that persons harboring mutant KRAS genotypes are at two fold risk of developing colorectal carcinoma than general population and the etiologic fraction (EF) of these mutant genotypes collectively accounted for 0.38, as shown in table 2.The results of the HRMA (amplification and melting curve) represented in figure 1 showed the melting curve results of only 35 samples. An advantage of performing HRMA analysis on a real time PCR machine with HRM capability, is that the PCR amplification and HRM analysis are performed in the one run and the results are available for analysis at the end of the run. While the figure 2 showed the difference between two types of KRAS mutation melting curve.

Table 1: KRAS genotypes in control and study groups.

KRAS genotype		Control group *n* = 20	Study group *n* = 40	*P* *
Wild	GC	15 (75.0 %)	24 (60.0 %)	Reference
Mutant	AC	3 (15.0 %)	8 (20.0 %)	0.724 NS
	AT	1 (5.0 %)	2 (5.0 %)	1.000 NS
	GT	1 (5.0 %)	6 (15.0 %)	0.394 NS

***n*: number of cases; *: Fischer exact test; NS: not significant**

Table 2: Mutant and wild type KRAS genotypes according to groups.

KRAS genotype	Control group *n* = 20	Study group *n* = 40	χ^2	*P* *	OR	95% CI	EF
Wild	15 (75.0 %)	24 (60.0 %)	1.319	0.251 NS	2.0	0.61 - 6.60	0.38
Mutant	5 (25.0 %)	16 (40.0 %)					

***n*: number of cases; *: chi-square test; NS: not significant; OR: Odds ratio; CI: confidence interval; EF: etiologic fraction.**

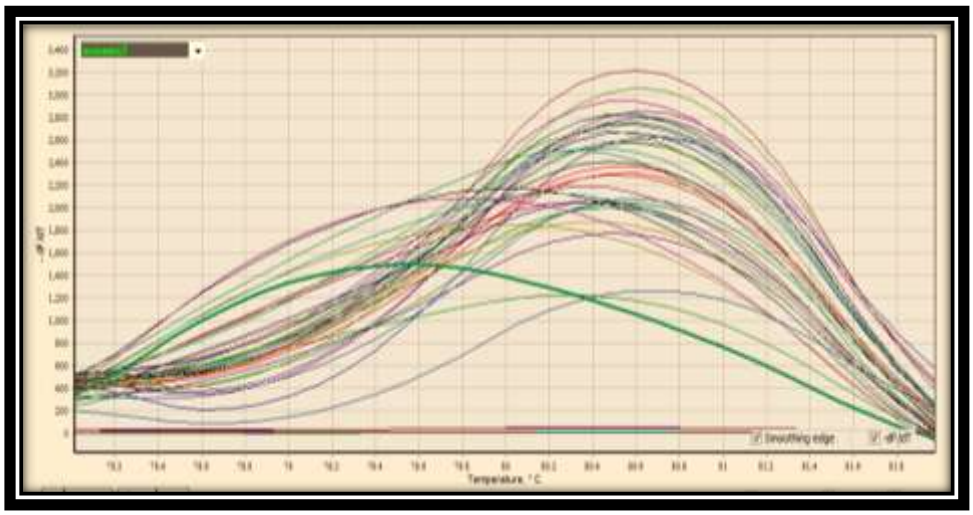

Figure (1); different melting peaks for mutation scanning of KRAS gene.

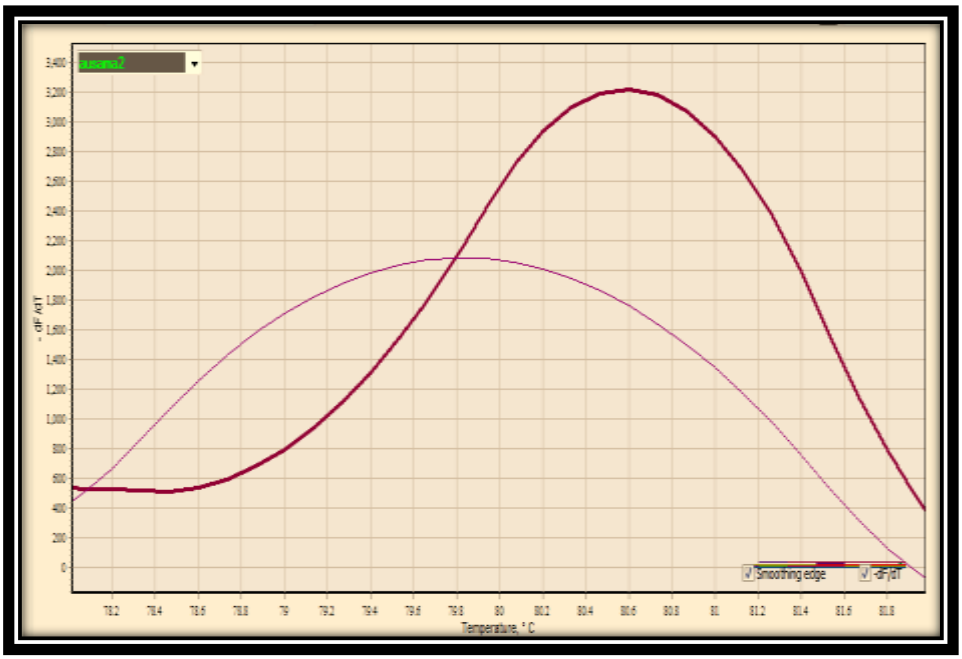

Figure (2); two types of melting curves (1) wild type (2) 12AC mutation.

In this study the aim is to develop a highly sensitive method for detecting somatic mutations of KRAS gene for genotyping CRC patients. The high resolution melting analysis technique (HRMA) applied to mutation scanning is often implemented in high-resolution format. Upon the completion of amplification, PCR products are subjected to the melting procedure in the presence of fluorescent probes. Characteristic changes in DNA melting curves indicate the presence of mismatched bases in the duplexes. and, therefore, the presence of mutations (3).

Since TaqMan probes are present in the incubation medium at both steps of the analysis (amplification and melting curve) the analysis can be carried out in the closed tube format. This is a 1.5–2-h assay in a single tube without any intermediate or additional procedures that minimizes not only time and labor expenditures, but also the probability of the cross contamination of samples, which is the most important, so The HRMA method is much more sensitive than Sanger sequencing (3). Our results agreed with (4,5) who revealed that codon 12 AC mutation were in a higher frequency in patients followed by the codon 13 mutation.

Conclusions

On the basis of the current study, significant over productions of all investigated parameters (KRAS mutations, CD33, IL17A) seems to have potential role in colorectal cancer progression and worse prognosis when compared with healthy people.

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