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Analysis of KRAS, BRAF and NRAS in Patients with Colorectal Cancer: the First Report of Western Iran

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Abstract **Background:** KRAS/NRAS/BRAF mutations are useful markers for predicting responses to anti-EGFR monoclonal antibodies in metastatic colorectal cancers. The aim of this study was to investigate the clinicopathological characteristics and distribution by tumor localization of KRAS mutations in metastatic colorectal cancer and analysis of NRAS and BRAF in the patients in Western Iran. **Materials and Methods:** Between May 2008 and November 2014, Thirty- three patients with metastatic or high risk CRC were included in our study. DNA was extracted by FFPE QIAGEN Kit and also KRAS/NRAS and BRAF *V600E* were analyzed using allele specific PCR primers and pyrosequencing. The overall survival for patients was plotted by GraphPad Prism 5 software. **Results:** The mean of age for patients at diagnosis was 57.67±13.20 years (range, 28-80 years), 19 patients (57.6%) were male. Of 33 patients, 9 patients (27.3%) were high risk and rest of patients had metastasis that metastasis was more to liver and lung, respectively. Of 33 patients, 21 patients (63.6%) have KRAS wild-type and 12 patients (27.3%) have KRAS mutation. Also, 5 samples of patients were checked for BRAF and NRAS. The mean overall survival for patients with metastatic colorectal cancer was 20 months. Location of tumor in 32 patients with metastatic colorectal cancer was left-side colon. **Conclusions:** NRAS/BRAF testing should be used together and with KRAS genotype to select patients who will likely benefit from anti-EGFR therapy and also location of tumor probably in patients with metastatic colorectal cancer in western Iran is more on left-side colon that it needs other studies with greater volume of patients.

Keywords: *KRAS, NRAS, overall survival, colorectal cancer*

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1. Introduction

Cancer is one of the major public health problems in the world. Globally, among common cancers, colorectal cancer is the fourth most common cancer in men and the third most common in women [1]. Activating mutation of KRAS plays a significant role in the pathogenesis of common human malignancies and molecular testing of KRAS mutation has emerged as an essential biomarker in the current practice of clinical oncology [2] *KRAS* mutations are useful markers for predicting responses to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies in metastatic colorectal cancers (mCRC). CRC patients with a *KRAS* mutation do not respond to treatment with cetuximab or panitumumab [3] Clinical data have proven that mutant RAS genes are negative predictive biomarkers and that patients with a *KRAS/NRAS* mutation do not benefit from an anti-EGFR-antibody-based therapy [4] and The role of *BRAF* in first-line treatment of mCRC is described as a negative prognostic marker, but not as a predictive marker in terms of anti-EGFR-antibody therapy [5]. BRAF mutant tumors are associated with poor outcome [6]. NRAS mutations occur in 3–5% of colorectal cancer [7] and 12 percent of KRAS wild-type colorectal cancer samples showed BRAF gene mutation [8]. RAS mutant mCRC exhibited a significantly higher cumulative incidence of lung, bone, and brain metastasis and on multivariate analysis was an independent predictor of involvement of these sites [9].

Some statistical reports demonstrated that the most common tumor location is the proximal colon, followed by the rectum [10]. In Iran, The rectum and sigmoid colon were the most frequent anatomical locations [11]. The most common histological type of colorectal carcinoma is moderately differentiated adenocarcinoma [12].

The aim of this study was to investigate the clinicopathological characteristics and distribution by tumor localization of KRAS point mutations in metastatic colorectal cancer and analysis of NRAS and BRAF in the patients in Western Iran.

2. Patients and Methods

Between of May 2008 and November 2014, Thirty-three patients with metastatic or high risk CRC were included in our study. We checked age, sex, tumor location, type of metastasis, KRAS/NRAS/BRAF (V600E) testing and overall survival (OS) in the patients.

2.1. Method for KRAS, BRAF and NRAS Mutations

DNA extracted by FFPE QIAGEN Kit and KRAS, BRAF (V600E) and NRAS were analyzed using allele specific PCR primers and pyrosequencing. The results have been double checked by high resolution melting analysis. Detection limit of this assay is five copies (codons 12, 13, 59, 61, 117 and 176) of mutations in whole genome. Every molecular test has a 0.5-1% error rate. This is due to rare molecular events and factors related to the preparation and analysis of samples.

KRAS mutations: G12D (c.35G>A), G12A (c.35G>C), G12V (c.35G>T), G12S (c.34G>A), G12R (c.34G>C), G12C (c.34G>T), G13D (c.38G>A), Q61K (c.181C >A), Q61L (c.182A>T), Q61R (c.182A>G), Q61H (c.183A>C), Q61H (c.183A>T), Q61E (c.181C>G), A59T (c.175G>A), A59G (c.176C>G), K117N (c.351A>C), K117N (c.351A>T), A146P (c.436G>C), A146T (c.436G>A) and A146V (c.437C>T)

NRAS mutations: G12A (c.35G>C), G12C (c.35G>T), G12S (c.34G>A), G12R (c.34G>C), G12V (c.34G>T), G12W (c.34G>T; c.36T.G), G12N (c.34_35GG>AA), G13D (c.38G>A), G13C (c.37G>T), G13V (c.38G>T), G13R (c.37G>C), G13E (c.37G>C), Q61K (c.181C >A), Q61L (c.182A>T), Q61R (c.182A>G), Q61H (c.183A>C), Q61H (c.183A>T), Q61E (c.181C>G), A59T (c.175G>A), A59G (c.176C>G), K117N (c.351A>T), A146P (c.436G>C), A146T (c.436G>A) and A146V (c.437C>T).

2.2. Statistical Analysis

The overall survival for patients was plotted by GraphPad Prism 5 software with Log-rank (Mantel-Cox) test that $P \leq 0.05$ was statistically significant.

3. Results

Table 1. Patient characteristics with colorectal cancer (n=33)

Variables	n (%)	Mean ±SD	Range
Age		57.67 ±13.20	28-80
Gender			
Male	19 (57.6)		
Female	14 (42.4)		
Tumor Location			
Rectum	13 (39.4)		
Sigmoid	11 (33.3)		
Descending Colon	5 (15.2)		
Rectosigmoid	3 (9.1)		
Cecum	1 (3)		
Type of Metastasis			
No Metastasis(High Risk)	9 (27.3)		
Liver	17 (51.6)		
Lung	2 (6.1)		
Lung + Liver	1 (3)		
Lung + Liver + Brain	1 (3)		
Liver + Small Intestinal	1 (3)		
Thyroid	1 (3)		
Peritoneum	1 (3)		
Kind of Pathology			
Invasive Adenocarcinoma	29 (87.9)		
Mucinous Adenocarcinoma	4 (12.1)		

Between May 2008 and November 2014, one-hundred eighty-six patients with CRC referred to our Clinic that Thirty- three patients had metastatic or high risk CRC and they were included in our study. The mean age for them at diagnosis was 57.67 ±13.20 years (range, 28-80 years) that 19 patients (57.6%) were male and 14 patients (42.4%) were female (Table 1). Location of tumors in patients was: rectum (39.4%), sigmoid (33.3%), descending colon (15.2%), rectosigmoid (9.1%) or cecum (3%).

Of 33 patients with colorectal cancer, 9 patients (27.3%) were stage III or high risk stage II and rest of patients had metastasis (stage IV) to liver (51.6%), lung (6.1%), lung + liver (3%), lung + liver + brain (3%), liver + small intestinal (3%), thyroid (3%) and peritoneum (3%). In all of patients, kind of pathology for colorectal cancer in 29 patients (87.9%) were invasive adenocarcinoma and 4 patients (12.1%) were mucinous adenocarcinoma.

The Table 2 shows RAS distribution in patients. 21 patients (63.6%) have KRAS wild-type, 9 patients (27.3%) have KRAS mutation in codon 12 and 3 patients have KRAS mutation in codon 13. Of 33 patients, 5 samples of patients were checked for BRAF and 13 samples for NRAS that all of them were BRAF wild-type and NRAS wild-type, respectively. Of 29 patients with invasive adenocarcinoma, 20 patients had metastasis to liver. For NRAS and BRAF testing, due to financial difficulties the patients, we couldn't test them in all of patients, but we try that do in the near future these tests in a lot of patients.

Table 2. *KRAS*, BRAF and NRAS distribution in patients with colorectal cancer (n=33)

Tests	n (%)
KRAS	
Wild-Type	21 (63.6)
Mutation in Codon 12	9 (27.3)
Mutation in Codon 13	3 (9.1)
BRAF	
Wild-Type	5
NRAS	
Wild-Type	13

The three-year OS of the patients with mCRC has been shown in Figure 1. The mean survival for the patients was 20 months with survival rate of 34.6% (17 deaths).

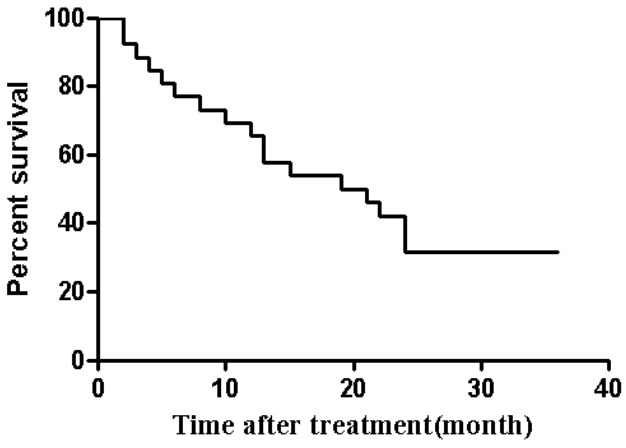

Figure 1. The 3-year overall survival for all of patients with metastatic colorectal cancer

The 3-year overall survival for patients with KRAS wild-type vs. KRAS mutations has been shown in Figure 2. The mean OS of the patients with KRAS wild-type was 19 months and for KRAS mutations was 24.5 months.

There was no significant correlation between the two groups (P>0.05).

Some of variables in the patients with NRAS wild-type or BRAF wild-type have been shown in Table 3. Time of follow up for patients with KRAS/NRAS wild-type was better than KRAS mutation and NRAS wild-type and also, in the patients with BRAF wild-type and KRAS mutation was better than KRAS/BRAF wild-type.

Table 3. The characteristics of patients with NRAS wild-type or BRAF wild-type

Age	Sex	Time of Follow up (month)	Mortality	KRAS	NRAS	BRAF
65	M	2	L	Wt	Wt	
54	M	3	L	Wt	Wt	
28	M	28	L	Wt	Wt	
60	F	36	L	Wt	Wt	
65	F	4	L	Wt	Wt	
43	M	22	L	Wt	Wt	
55	F	60	L	Wt	Wt	
58	M	2	L	Wt	Wt	
74	F	4	L	Mu	Wt	
53	M	8	L	Mu	Wt	
48	F	77	L	Mu	Wt	
55	M	2	L	Mu	Wt	
69	M	10	L	Mu	Wt	
61	F	4	D	Wt		Wt
38	F	13	D	Wt		Wt
77	M	39	D	Mu		Wt
51	F	47	L	Mu		Wt
73	F	22	D	Mu		Wt

Mu: mutation Wt: Wild-type L: Live D: Dead

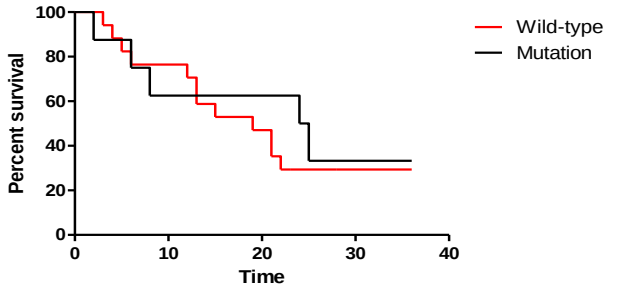

Figure 2. The 3-year overall survival for patients with KRAS wild-type vs. KRAS mutations

4. Discussion

CRC is the third most common neoplastic disease worldwide. It is one of the leading causes of cancer mortality, accounting for about 10% of all cancer deaths, with approximately 40%-50% of all cases diagnosed as metastatic [13].

The addition of monoclonal antibodies that bind the vascular endothelial growth factor and the EGFR to chemotherapy regimens in mCRC has been shown to be effective, thereby increasing treatment options [14,15]. Advances in the treatment of metastatic colorectal cancer (mCRC) over the last 20 years have improved OS from a median of 10 months to approximately 24 months [1]. Differently from KRAS and BRAF mutations, the role of NRAS mutations as prognostic and predictive markers in metastat mCRC has been investigated to a less extent [7].

KRAS mutations were reported to be more frequent in right-colon tumors by Bleeker *et al.* [16] but in left-colon tumors by Zulhabri *et al.* [17] A study [18] showed that KRAS mutations are more frequent in sigmoid and rectal adenocarcinomas and also other study [19] reported that KRAS mutation in rectosigmoid tumours is than that of colon-localized tumours. A number of studies [7,8,13,18,20,21] reported The most prevalent mutations were in codon 12 compared with codon 13. Our Study confirms these results that KRAS mutations are found to be higher in left-sided colon cancer and codon 12 but a number of studies [22,23,24] reported that KRAS mutation is higher in patients with right-sided colon cancer.

In a study [8] of 109 patients with CRC, 42% had KRAS mutation and also Gunal A et al. [25] with survey of 145 CRC patients concluded that 55 patients (37.9%) had KRAS mutations and in our study in mCRC patients was 36.4% but Montomoli J et al. [20] reported that The overall prevalence of KRAS mutations in mCRC patients was 55%.

Montomoli J et al. [20] identified 106 mCRC patients with median age at CRC diagnosis: 61 years; 64% were males and n this study, mean age at diagnosis for patients was 57.67 years, 57.6% were male.

The 1-, 2-, and 5-year OS rate after colorectal cancer diagnosis for patients was 91%, 68%, and 25%, respectively. [20] In our study, 3-year OS rate was 34.6% and median survival was 20 months.

The most common sites of colon cancer metastasis are the regional lymph nodes, liver, lung, bone and brain [26]. Field K et al. [27] reported about 40%-50% of CRC patients develop metastases during their clinical history, and 80%-90% of them have liver secondary lesions and in other study [28] of 15 patients with KRAS wild type mCRC, The distribution of metastatic sites was: Liver (12; 80%), distant lymph nodes (7; 47%), peritoneum (6; 40%), lung (5; 33%), spleen (1; 7%) and bone (1; 7%). Of 21 patients with KRAS wild type mCRC in our study, metastasis to liver, Lung and peritoneum were 52.3%, 9.5% and 4.7%, respectively.

De Roock W at al. [29] reported that median OS was significantly better in KRAS Wild-type versus mutants (43.0 versus 27.3 weeks) but in this study, median 3-year OS was not statistically significant between KRAS wild-type and KRAS mutations (19 vs. 24.5 months, P>0.05) and also Kim ST et al. [30] analyzed that the response rate, PFS and OS did not differ between KRA wild-type and KRAS mutations mCRC patients treated with chemotherapy alone. These findings suggested that KRAS

is not a prognostic marker for CRC. Therefore, NRAS/BRAF mutation probably is effective in treatment of patients with KRAS wild-type and in patients with KRAS wild-type should be specified NRAS/BRAF testing to determine which patients will benefit from anti-EGFR therapy [31]. The Table 3 shows that of 5 patients with BRAF wild-type, 3 patients were KRAS mutation that had the mean follow up better than 2 patients with KRAS wild-type. Also, the mean follow up for KRAS/NRAS wild-type was better than KRAS mutation and NRAS wild-type.

5. Conclusions

Results suggest that NRAS/BRAF testing should be used together and with KRAS genotype to select patients who will likely benefit from anti-EGFR therapy. Also, location of tumor probably in patients with metastatic colorectal cancer in Western Iran is more on left-side colon that it needs other studies with greater volume of patients.

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