RESEARCH ARTICLE

Clinicopathological Features and Survival Rate of Colorectal Adenocarcinoma Patients with and without a KRAS Mutation: a Five-Year Study in Yazd, Iran

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Abstract

Background: By some estimates, colorectal carcinoma is the third most common cancer worldwide. The most appropriate method of treatment, especially of its metastatic form, is determined based on KRAS status. The present study was conducted on patients with colorectal cancer positive or neagtive for a KRAS mutation in terms of survival rate and the response to treatment. Materials and Methods: Medical records of all cases with colorectal cancer hospitalized from 2010 to 2015 and with KRAS testing results were studied. Data such as gender, age, tumor (size, grade, location, stage), treatment type, KRAS status and survival were considered as variables. Survival analysis was performed using the Kaplan-Meier method and Log-rank test. Statistical significance level was defined as P value <0.05. Results: Out of 90 patients, 55 (61.2%) were male and 35(38.8%) were female with the age range of 22-87 years. The overall disease specific survival was 53±3 (Mean ± SE) months with 95%CI:47-60, and there were statistically significant differences between the mean survival rate with tumor stage and the response to treatment (log rank test, PV=0.007 and PV=0.001) respectively. The risk of mortality was 2.02 times higher in patients with mutant KRAS compared to those with the wild type of the gene; however, this difference was not statistically significant (OR=2.016; 95%CI: 0.68-5.9; PV=0.197). Conclusions: In our study the overall 5-year disease specific survival rate was low as compared to similar studies elsewhere. Significant correlations were found between survival time with treatment type and tumor stage.

Keywords: Colorectal carcinoma - survival - KRAS - treatment type - tumor stage

Asian Pac J Cancer Prev, **17 (7)**, 3417-3422

Introduction

Colorectal cancer is the third most common type of cancer among men and women worldwide (world cancer report., 2014; Ferlay et al., 2014). In Iran, colorectal cancer is the third most common cancer among men and the fourth most common among women (Shaib et al., 2013; Cunningham et al., 2010). The prevalence of this cancer has been reported as 1.4 times higher among men than among women (world cancer., 2014; SEER., 2014).

The 5-year survival rate of colorectal cancer varies in different countries, and this rate has been reported in less than 60% of the cases in Europe and in about 65% of the cases in the United States (SEER., 2014; Howlader et al., 2012). The mean survival rate for this cancer seems to be directly correlated with the tumor stage at the time of the patient's hospital admission, as in stage 0 of the disease (TNM stage Tis, N0, M0), the 5-year survival rate is reported in 100% of the cases, while it drops below 70.8% with lymph node involvement (stage III A, B, C) and less than 13% in stage IV (A, B) of the disease (Howlader et al., 2012; Kolahdoozan et al., 2010; Dolatkhah 2015). Colorectal cancer tends to affect people over the age of 50 (world cancer report., 2014).

Its treatment options include surgery, chemotherapy, radiotherapy, neo-adjuvant therapy and targeted therapy (Mohammadipanah 2015). Epidermal growth factor receptor is a membrane bound receptor tyrosine kinase(RTK), with an important role in cancers initiation and progression. Now today the Kirsten rat sarcoma-2 viral (v-Ki-ras2) oncogene homologue (KRAS) protein has been well known as a target of therapy by anti epidermal

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growth factor receptor inhibitor, which in humans is encoded by the KRAS gene (located on the short arm of chromosome 12 at position 12.1)) (Mohammadipanah 2015; McGrath et al., 1983; Misale et al., 2012; Allegra et al., 2016). This proto-oncogene could be mutated in some patients with colorectal carcinoma (30-50%) and the result of this mutation is lack of responsiveness to treatment by anti epidermal growth factor receptor inhibitors (Kito and Yamazaki 2015; Deng et al., 2015). In colorectal carcinoma the presence or absence of KRAS mutations has become the main focus of researchers and clinicians in this area and has affected their decisions about the proper types of treatment available to this group of patients and, in recent years, anti-epidermal growth factor receptor treatments are no longer used in the treatment of patients with mutated KRAS (30-40% of the cases) (Yaeger et al., 2015; Vauthey et al., 2013). However, given the different reports on the effect of this treatment on the prognosis of colorectal cancer, the present study was conducted on colorectal cancer patients to find out the differences in survival and recovery rate based on KRAS mutation status.

Materials and Methods

In this retrospective study hospital folders of all colorectal cancer patients admitted to oncology and pathology wards affiliated with Shahid Sadoughi University of Medical Sciences from March 2010 to April 2015 in Yazd, Iran were reviewed. The study was approved by the Research Ethics Committee of the school of Medicine. Although the study only included a review of patient's records obtained as part of the routine medical care, patients consent forms were obtained when they were first hospitalized. It is a general policy of Shahid Sadoughi affiliated health care centers to obtain a written consent form from the patients upon their freewill, agreeing that their medical records can be used for research purposes. Patient's data confidentiality was maintained and no data regarding patient's personal information were disclosed. The inclusion criteria for entering the study was: all the patients diagnosed with colorectal carcinoma who had a record in the pathology and oncology departments with KRAS test result. All the records that had missing data (including test results) or a written consent form was not obtained, were excluded from the study. In result 90 records were identified and included in the study.

Patient's data were extracted using a checklist which included: age, gender, date of reference and diagnosis, date of death, KRAS status, size and place of tumor, tumor stage, patient's treatment type and grade of tumor. Patient's telephone number and place of residence were also extracted for further follow-ups and assessing patient's survival rates.

Definition of variables

The site of tumor involvement was divided into four regions including: the rectum, right colon, left colon and the transverse colon. Tumors were classified into 3 groups divided by size, including a group less than 5 cm (Group 1), 5-10 cm tumors (Group 2) and a group with tumors larger than 10 cm (Group 3). The grading of the tumors was done based on their differentiation pattern including: Well (>95% gland forming), Moderate (50-95% gland forming) and Poor (0-49% gland forming). Tumor staging was performed by using TNM staging system of colorectal carcinoma (TNM/AJCC-7th Edition) into stages: I, II, III, IV and their subgroups. The KRAS and NRAS status results were extracted from patient's hospital records which was divided into two groups including: Wild-type (KRAS and NRAS) and Mutant (KRAS and NRAS) group by using the PCR method (PARTOLAB Pathology Laboratory Molecular Diagnostic Division, Tehran, Iran).

Treatment type

Patients treatment method was extracted from their hospital records which was based on the presence or absence of metastasis; as patients without metastasis were treated by FOLFOX regimen (Folinic acid, Fluorouracil, Oxaliplatin) (Treatment type 1), and patients with metastasis were treated with respect to their KRAS type, i.e. patients with the wild type of the gene were treated by a combination of FOLFOX and Cetuximab (Treatment Type 2) , while patients with mutant KRAS had received a combination of FOLFOX and Bevacizumab as their select treatment (Treatment type 3). FOLFIRI treatment containing Folinic acid, Fluorouracil and Irinotecan was used for patients who experienced complications related to Oxaliplatin. For evaluation of response to treatment, patients were also classified into two groups based on its effect on reduction of the patient's serum CEA levels, elimination of metastasis and tumor shrinkage, including: group 1, showing an appropriate response to treatment, and group 2, showing a lack of response to treatment.

Following pathology and oncology evaluation, patients were contacted via telephone to acquire information regarding their current status. Follow-up time was the interval between diagnosis time and the time of death or last contact with the patient.

Statistical analysis

The data analysis was done using the SPSS software version 17 for windows (SPSS Inc., Chicago, IL, USA). Patient's data were compared and analyzed using the Chi-square test and for calculating the survival fraction and assessing the effect of different factors on the survival of the patients, the Kaplan–Meier and the Log rank tests were used, respectively. The descriptive data of the patients were compared using their frequency, mean and standard deviation. The odds ratio was measured with a Confidence interval (CI) of 95% to estimate the risk of mortality based on the KRAS status. A two-tailed p-value of less than 0.05 was considered as statistically significant.

Results

Among the 90 cases whom enrolled in this study, 55 (61.2%) were male and 35 (38.8) were female, with the age range of of 22-87 and (mean±SD) age of 56±14 years. The male to female ratio was approximately 1.6:1. Baseline characteristics of 90 patients with colorectal adenocarcinoma are shown in Table 1. As for the KRAS status, 54 (60%) had a wild type KRAS and 36 (40%) had

Table 1. Patient Characteristics Based on KRAS Status

Characteristics			KRAS status		Total N (%)	P value
			Wild N (%)	Mutant N (%)		
Age	Female	22-54	12(63.2)	7(36.8)	19(100)	0.968
		55-87	10(62.5)	6(37.5)	16(100)	
	Male	22-54	17(68)	8(32)	25(100)	0.178
		55-87	15(50)	15(50)	30(100)	
Gender	Female		22 (62.9)	13 (37.1)	35 (100)	0.659
	Male		32 (58.2)	23 (41.8)	55 (100)	
Tumor size	1		30 (62.5)	18 (37.5)	48 (100)	0.85
	2		11 (68.8)	5 (31.3)	16 (100)	
	3		1 (100)	0 (0)	1 (100)	
Tumor location	Rectum		15 (71.4)	6 (28.6)	21 (100)	0.591
	Right colon		11 (52.4)	10 (47.6)	21 (100)	
	Transverse		7 (53.8)	6 (46.2)	13 (100)	
	Left colon		15 (62.5)	9 (37.5)	24 (100)	
Stage	1		2 (100)	0 (0)	2 (100)	0.309
	2		28 (68.3)	13 (31.7)	41 (100)	
	3		12 (60)	8 (40)	20 (100)	
	4		10 (47.6)	11(52.4)	21 (100)	
Grade	1		20 (62.5)	12 (37.5)	32 (100)	0.813
	2		19 (54.3)	16 (45.7)	35 (100)	
	3		1 (50.0)	1 (50.0)	2 (100)	
Metastasis	Yes		21(53.8)	18 (46.2)	39 (100)	0.348
	No		30 (63.8)	17 (36.2)	47 (100)	
Treatment type	1		30 (62.5)	18 (37.5)	48 (100)	0.001*
	2		22 (100)	0 (0)	22 (100)	
	3		0 (0)	18 (100)	18 (100)	
Out come	Yes		37 (75.5)	19 (59.1)	56 (100)	0.124
	No		12 (24.5)	13 (40.6)	25 (100)	

* Chi-square test

Table 2. Univariate Prognostic Analysis of Overall Survival

Characteristics		Survival time (mean ±SE)	95%CI	Survival time (Median ±SE)	95% CI	Total	Number events (N)	Number censored N (%)	P value*
Gender	Female	53±6	42-64	59±0	-	31	6	25 (80.6)	0.825
	Male	51±3	44-57	62±14	34-90	51	11	40 (78.4)	
Age	22-54	62±4	55-69	-	-	38	4	34 (89.5)	0.13
	55-87	47±4	40-54	59±10	40-78	44	13	31 (70.4)	
Tumor location	Rectum	50±7	36-63	59±24	13-105	20	5	15 (75)	0.815
	Right colon	49±6	36-61	-	-	21	4	17 (80.9)	
	Transverse	41±5	32-51	-	-	11	2	9 (81.8)	
	Left colon	53±5	43-63	-	-	22	3	19 (86.4)	
Stage	1	-	-	-	-	2	0	2 (100)	0.007
	2	56±3	49-63	-	-	39	4	35 (89.7)	
	3	46±2	41-50			19	2	17 (89.5)	
	4	38±6	26-51	62±0	-	17	9	8 (47.1)	
Grade	1	49±4	40-57	59±0	-	31	6	25 (80.6)	0.054
	2	38±3	32-43	-	-	33	7	26 (78.8)	
	3	8±1	10-May	6±0	-	2	1	1 (50)	

* Log rank test

Table 3. Univariate Analyses of Survival Outcomes by Kras Status, Metastasis and Treatment Type

Characteristics		Survival time (mean ±SE)	95%CI	Survival time (median ±SE)	95%CI	Total (N)	Number of events (N)	Number of censored (%)	P value
Metastasis	Yes	40±5	31-50	42±15	13-71	35	16	19 (54.3)	0.001*
	No	61±2	57-65	-	-	46	1	45 (97.8)	
KRAS status	Wild	56±4	48-65	-	-	49	8	41 (83.7)	0.155
	Mutant	47±5	37-56	62±0	-	33	9	24 (72.7)	
Treatment type	1	61±2	57-65	-	-	44	1	43 (97.7)	0.001*
	2	46±7	33-58	59±19	22-96	20	7	13 (65)	
	3	35±7	22-49	27±8	Dec-42	16	9	7 (43.7)	
Outcome	Yes	-	-	-	-	53	0	53 (100)	0.001*
	No	29±5	19-38	22±6	Oct-34	24	17	7 (29)	

*Log rank test

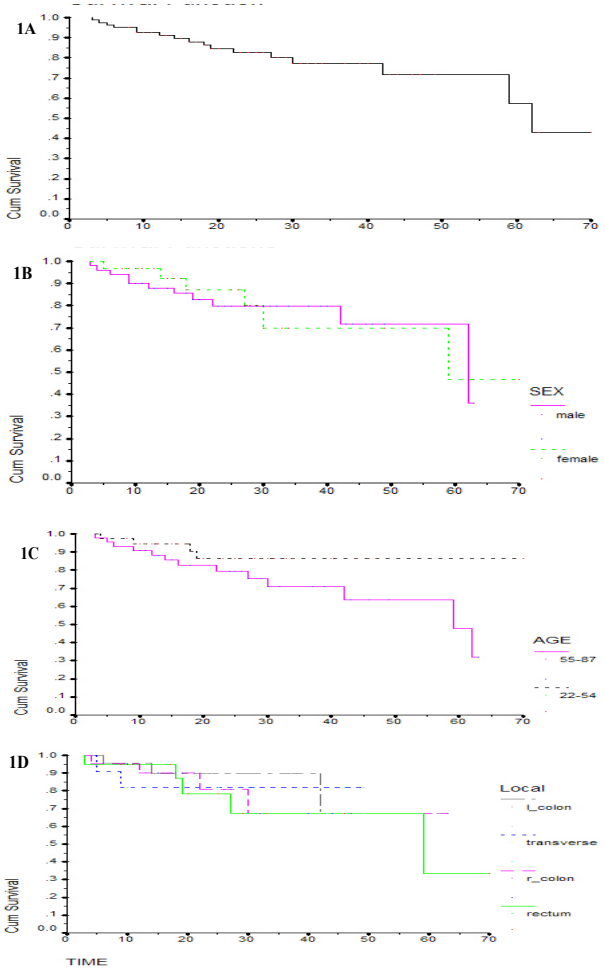

Figure 1. Kaplan–Meier Survival Estimates for Colorectal Carcinoma Patients. A) Overall; B) Stratified by sex; C) Stratified by age; D) Stratified by location

a mutant KRAS. No statistical difference was observed between the patients with mutant KRAS and those with the wild type of the gene in terms of age and gender (Table1). Wild type KRAS was more expressed in tumors located in rectum (71.4%), in comparison to other anatomical sites involvement(not statistically significant). Patients with metastasis had more expression of mutant type of KRAS (46.2%) in comparison to patients who had not metastasis, but this was not significant. The mean survival rate was 53±3 months (Mean±SE) with a (95%CI, 47-60) in the samples examined. The Kaplan-Meier curve showed the probability of survival rate have decreased with a gentle gradient for 57 months after the initial diagnosis, and then there's a steep decrease until month 62 and then plateaued (Figure 1A). According to Table 2, significant correlation was found between survival time and tumor stage (PV=0.007). The survival time had not significant association with patients age, gender, tumor location, grade and type of the gene (Table 3, Figures 1, 2). Patients with metastasis who expressed mutant KRAS (treatment type 3) had a shorter survival time than those with wild type KRAS (treatment type 2) and patients without metastasis (treatment type 1) (PV=0.0001) (Figure 3). Based on the odds ratio (OR), the risk of mortality was found to be 2.02 times higher in patients with mutant KRAS than in those with the wild type of the gene; however, this difference was not statistically significant (PV>0.05).

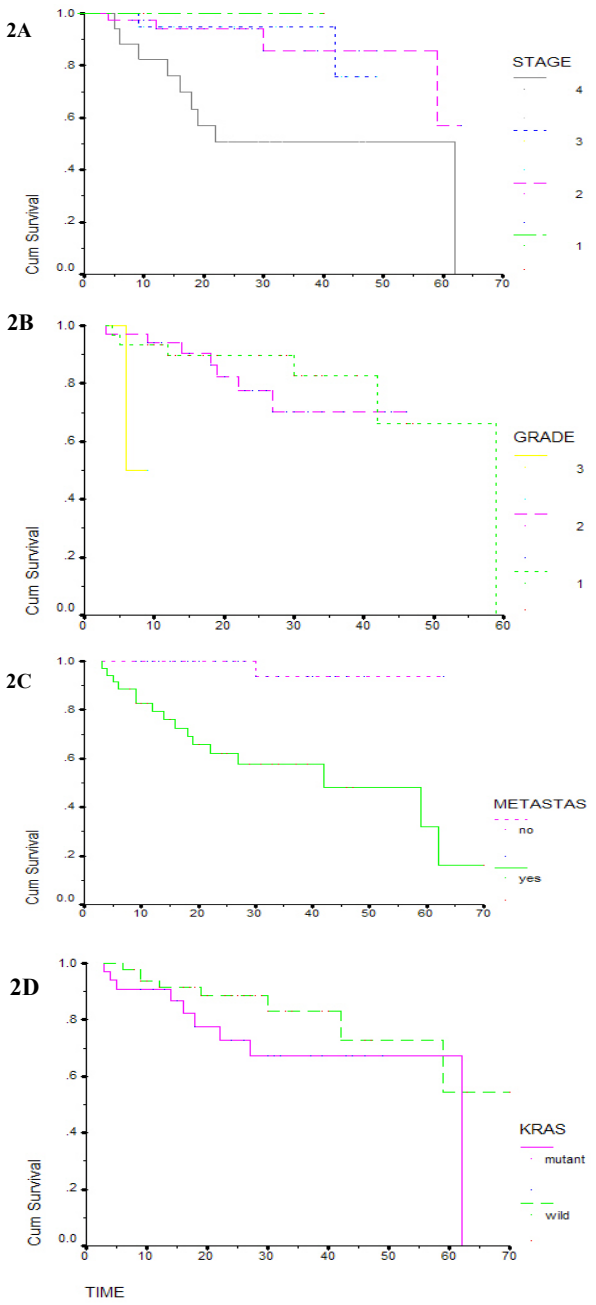

Figure 2. Kaplan–Meier Survival Estimates for Colorectal Carcinoma Patients. A) Stratified by stage (I, II, III, IV); B) Stratified by grades; C) Stratified by metastasis status; D) Stratified by KRAS status

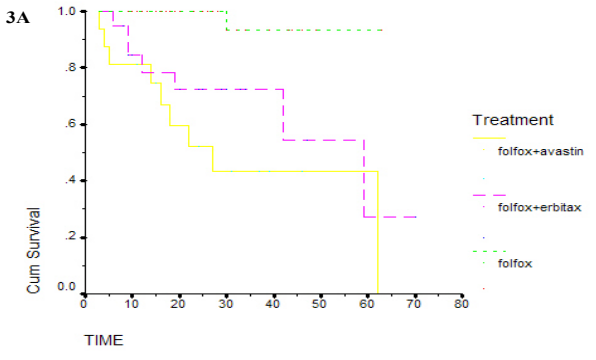

Figure 3. Kaplan–Meier Survival Estimates for Stratified by Treatment Type

Discussion

The present study conducted a statistical analysis of the clinicopathological findings of colorectal adenocarcinoma patients and relationship between KRAS status and the variables such as (gender, age, tumor site, tumor grade, stage of the disease, type of treatment, mean survival rate and prognosis). The only variable significantly related to KRAS status was the stage of the disease. Moreover, a mutant KRAS indicated a worse prognosis for patients than a wild-type KRAS and was also associated with a poorer response to treatment.

Based on literatures, about 30-50% of patients with colorectal cancer appear to have mutant KRAS (Howlader et al., 2012; Kolahdoozan et al., 2010; Dolatkhah 2015). Therefore, the majority of patients respond appropriately to anti-epidermal growth factor receptor treatments (Breivik et al., 1994; Liu X et al., 2011; Arrington 2012; Phipps et al., 2013; Phipps et al., 2015). In our study about 40% of the patients had the mutant form of KRAS, which is consistent with the findings of similar studies (Dolatkhah et al., 2015; Mohammadianpanah et al., 2015; McGrath 1983). In previous studies, the frequency of mutant KRAS expression was almost equal among men and women and no statistically significant differences were reported in the expression of mutant KRAS by gender, which is compatible with the results of the present study (Vauthey et al., 2013). Kito et al., 2015. reported significantly more common KRAS mutations in tumors of the rectum than in tumors of other anatomic regions of the colon.

In the present study, although the expression of mutant KRAS was more common in right colon than in the other regions, the difference was not statistically significant. Previous studies have reported mutant KRAS to be correlated with tumor grade, as the majority of tumors with mutant KRAS were grade 2 (moderately differentiated) (Siyar et al., 2015). In the present study, the highest frequency of mutant KRAS was observed in the moderately differentiated tumors, although this difference was not statistically significant which could be due to small sample size of our study. Tumors with mutant KRAS are diagnosed at more advanced stages, while most tumors diagnosed at early stages have wild-type KRAS. Chang et al., 2014. reported 44% of patients with metastatic colorectal cancer to have KRAS mutations and also found no differences in survival rate between patients with mutant KRAS and those with wild-type KRAS.

In the present study, the frequency of the mutant type of the gene was higher in patients with the metastatic form of the cancer, although no significant relationship was found between gene type and metastasis. In terms of survival, previous studies have reported that about 40-60% of patients with wild-type KRAS do not respond appropriately to this type of treatment, as they also have mutant BRAF genes (Koike et al., 2014; Yammaz et al., 2015). Anti-EGFR therapy, such as a combination of FOLFOX, Panitumumab and Cetuximab, appears to be associated with a good prognosis for patients with wild-type KRAS; patients with this type of the gene also appear to attain a better prognosis when treated with a combination of Cetuximab and FOLFIRI (Rui et al., 2015; Selcukbiricik et al., 2013). Patients who have used combination therapies have responded better to treatments than those who have used one-drug regimens (Chang et al., 2014; Yanmaz et al., 2015). According to a study conducted by Yoon et al 2014. KRAS mutations are an independent predictive factor for survival as the mutant type of KRAS should be considered a negative predictive factor for patients with metastatic colorectal cancer. In the present study, the risk of mortality was 2.02 higher in patients with the mutant type of the gene than in those who had the wild type of the gene; however, this difference was not statistically significant. Patients with metastatic colorectal cancer who have wild-type KRAS seem to respond more appropriately to a combination treatment with FOLFOX and Cetoximab, as this treatment is associated with a better progression-free survival and overall survival in them compared to those who have the mutant type of the gene and have received the same treatment (Deng et al., 2015). Selcukbiricik et al. (2013) and Kadowaki et al. (2015), found that KRAS and BRAF mutations cannot be considered prognostic factors in patients with metastatic colorectal cancer (Li et al., 2012; Kadowaki et al., 2015). They concluded other factors, such as other mutation subtypes, can be used as strong prognostic factors.

The present study found a significant relationship between treatment regimens and survival rate, as mortality rates were noticed to be higher in patients who received treatment type 3 than in those who received the other two types of treatment. However, this significant difference was not due to the type of treatment regimen, rather to the progression of the disease and its metastasis in the patients. Moreover, this treatment regimen was used only for patients who had the metastatic form of the cancer, and not for patients with wild-type KRAS. Nevertheless, this treatment regimen did not reduce mortality rates. Consequently, survival rate can be said to be more related to the stage of the disease rather than KRAS type. Loupakis et al. in 2015 reported that patients with right colon cancer have a worse prognosis than patients with left colon cancer (Loupakis et al., 2015). The present study also found a higher mean survival rate in patients with tumors in their left colon than in those with tumors in their other anatomic regions; however, this difference was not reported to be statistically significant.

In conclusion, in our study, patients with metastasis who expressed wild type KRAS and were treated by combination of Folfax and Cetuximab had a better survival compared to patients with metastasis expressed mutant type of KRAS and were treated by Folfax and Bevacizumab. Also in our study, the survival time was significantly correlated with tumor stage.

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