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Original Research Article

The survival and KRAS/BRAF testing in colorectal cancer: areview on theliteratureof Iran

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Abstract: Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer death and incidence rates of CRC are highest in Australia, New Zealand, Europe, and North America and lowest in Africa and South-Central Asia. The aim of this study wasa retrospective analysis of patients hospitalized in the Clinic of Hematology and Oncology, Kermanshah, Iran, between 2002 and 2014. One hundred eighty-six CRC patients referred to our Clinic and data analysis for sex and age was performed using IBM SPSS.v19 and also survival was plotted by Kaplan-Meier plot and Log-rank test in Graph Pad prism 5 Software in a five-year period with two-year follow-up and DNA extracted by FFPE QIAGEN kit and KRAS were analyzed by applying allele-specific PCR X7 primers (ARMS method) and DXN scorpion and ARMS assay for detection of BRAF mutation V600E and pyrosequencing. The mean age at diagnosis was 54.27±13.24 years (range, 22 to 84 years) that 55.4% were males. The 5-year survival rate and mean were 84.61% and 41 months, respectively. Of 186 patients with CRC, 24 patients (12.9%) had metastatic CRC (62.5% KRAS wild type, 20.8% KRAS mutation in codon 12 and etc.). In conclusion, there was no the relationship between sex and age in patients, but the survival rate for females was higher than males and it was statistically significant (P<0.05) and also KRAS wild- type is more than KRAS mutations in patients with metastatic CRC in western Iran.
Keywords:BRAF, Colorectal Cancer, KRAS, Survival

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second most common cancer in females. Incidence rates of CRC are highest in Australia, New Zealand, Europe, and North America and lowest in Africa and South-Central Asia [1]. Nonetheless, it has been recognized that the risk of CRC of immigrants will gradually increase over time, attributed to the adoption of the western lifestyle including diet and exercise [2]. In Europe the five-year survival for CRC is less than 60%. In the developed world about a third of people who get the disease die from it [3]. KRAS, NRAS, or BRAF mutations can all activate the RAS-RAF-MAPK pathway, which is downstream of EGFR and The hypothesis is that KRAS mutation activates the RAS/MAPK signaling pathway downstream of EGFR independently of ligand binding to the receptor The detection of BRAF mutations is currently included in some clinical laboratory protocols, although it has not been established as routine clinical practice. BRAF is a protein member of the *RAF* family (RAF1, BRAF, ARAF), also regulated by RAS binding [4].

This study aims to evaluate the CRC status among a group of 186 patients in western Iran on a 12-year period. Additionally, age-sex correlation, comparative study on survival (considering other Asian countries), KRAS mutations and BRAF wild-type were documented.

MATERIALS AND METHODS

Patients

In our study, 183 patients with CRC referred to Clinic of Hematology- Oncology, Kermanshah, Iran, between 2002 and 2014. For all patients were calculated sex, age, survival and position of tumor in the first stepofdiagnosing of cancer.

DNA extracted by FFPE QIAGEN kit and KRAS were analyzed by applying allele-specific PCR X7 primers (ARMS method) and DXN scorpion and ARMS assay for detection of BRAF mutation V600E

and pyrosequencing. The results have been double checked by high resolution melting analysis. Detection limit of these assays is five copies of mutations in all genome. Mutations screened as: p.Gly12Asp (c.35G > A), p.Gly12Ala (c.35G > C), p.Gly12Val (c.35C > T), p.Gly12Ser (c.34G > A), p.Gly12Arg (c.34G > C), p.Gly12Cys (c.34G > T) and p.Gly13Asp (c.38G > A). For pathology report, the specimen is received in formalin, consists multiple tiny fragments of creamy soft tissue totally measuring 0.5 cm in greatest diameter.

Statistical Analysis

Data analysis for sex and age was performed using IBM SPSS v.19 software. The p-value was calculated with T- test that P<0.05 was significant. Overall survival (OS) was calculated as the time from diagnosis to death or last contact. Curve of the OS was plotted by Kaplan-Meier plot in Graph Pad prism 5 Software in a five-year period (March 2009 – March 2014) with completing a 2-year period and curves of OS for male and female were compared by log-rank test.

RESULTS

The mean of age at diagnosis for 186 patients with CRC was 54.27±13.24 years (range: 22 to 84), that 103 patients (55.4%) were males and 83 patients (44.6%) were females (**Table 1**). There wasn't significant difference between sex and age in patients (P>0.05). In the patients, 61 cases (32.8%) had a primary tumor in the location of sigmoid, 45 cases (24.2%) in rectum,32 cases (17.2%) in ascending colon, 22 cases (11.8%) in rectosigmoid, 14 cases (7.5%) in cecum, 8 cases (4.3%) in descending colon and 4 cases (2.2%) in transverse colon (**Table 1**).

Table 1: Baseline characteristics in patients with colorectal cancer (n=186)

Characteristic	Mean ± SD	n (%)	P-value
Age	54.27±13.24		P>0.05*
Sex			
Male		103(55.4)	
Female		83(44.6)	
Location of tumor			
Sigmoid		61(32.8)	
Rectum		45(24.2)	
Ascending colon		32(17.2)	
Recto sigmoid		22(11.8)	
Cecum		14(7.5)	
Descending colon		8(4.3)	
Transverse colon		4(2.2)	

* T-test

The **Figure 1** shows that CRC in men in younger ages (<50 years) and older ages (>60 years) is more than women, but in the middleages (50-59 years) in women is more. Also, it is determined that the number of patients with >70 and <40 is less and more of the patients have middle ages.

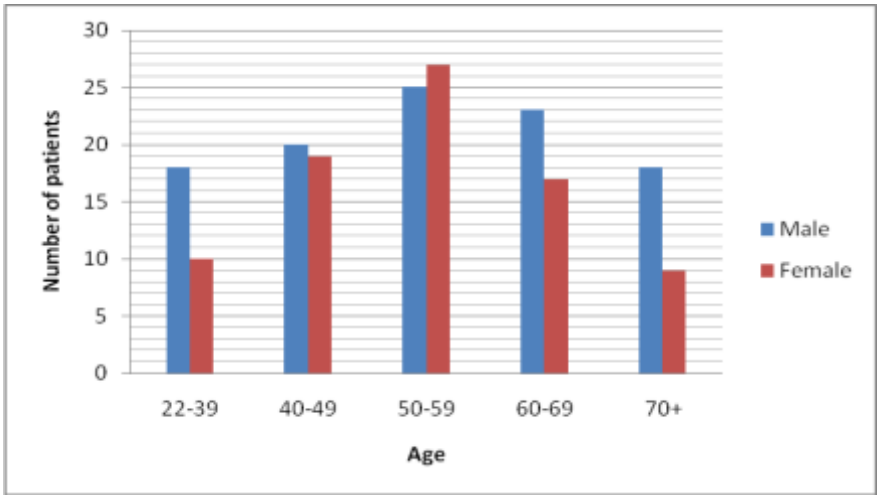

Fig 1: Comparison of age and sex with patients with colorectal cancer (n=186)

The 5-year OS (March 2009 – March 2014) for all of patients has been shown in **Figure 2**. Of 186 patients with CRC, 30 patients died and 36 patients were lost to follow-up before completing a two-year period and should therefore be excluded from the analysis. The median OS was 37.4 months and survival rate was 75 %.

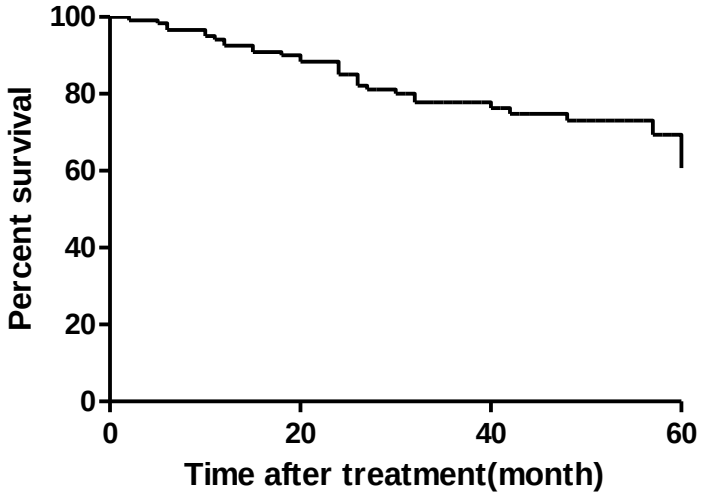

Fig 2: The 5-year overall survival for patients with colorectal cancer

The **Figure 3** shows OS rate for men comparing with women. There is a significant difference between sex and OS rate (P<0.05, 95% CI of ratio 1.04- 4.56, Hazard Ratio (HR) 2.18).

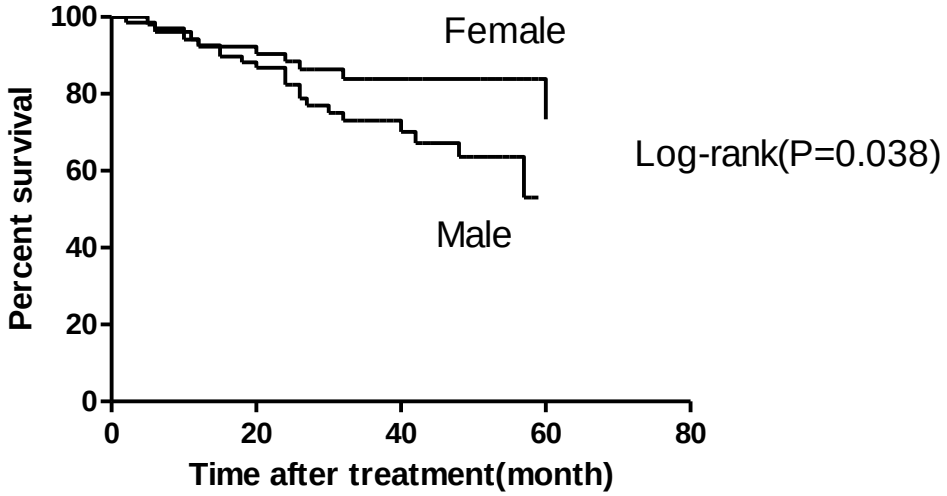

Fig 3: The 5-year overall survival for patients with colorectal cancer by sex

We analyzed KRAS mutations, KRAS and BRAF wild-type in patients with metastatic CRC. Of 186 patients with CRC, 24 patients (12.9%) had metastatic CRC(15/24 or 62.5% KRAS wild- type, 3/24 or12.5% KRAS wild-type + BRAF wild-type, 5/24 or 20.8% KRAS mutation in codon 12 and 1/24 or 4.2% KRAS mutation in codon 13)(**Table 2**). In patients with KRAS wild- type, 7 patients were females and 8 patients were males and the mean age was 55.9 years. The tumor location for them was 40% in sigmoid, 20% rectosigmoid, 13.3% ascending colon, 13.3% descending colon, 6.7% rectum and 6.7% transverse colon.

Three patients with KRAS wild-type + BRAF wild-type were females that 2 patients after 16 months and 20 months from diagnosis of CRC died. In 6 patients with KRAS mutations, the mean of age was 72 years that 5 patients (83.3%) were males.

Table 2: KRAS mutations, KRAS wild-type and BRAF wild-type distribution in patients with colorectal cancer

Gene	Sex	Age(year)	Tumor Location
KRAS wild type	Female	48	Rectosigmoid
KRAS wild type	Male	63	Transverse Colon
KRAS wild type	Female	80	Sigmoid
KRAS wild type	Female	37	Descending Colon
KRAS wild type	Female	52	Rectum
KRAS wild type	Male	33	Sigmoid
KRAS wild type	Male	56	Rectosigmoid
KRAS wild type	Male	59	Rectosigmoid
KRAS wild type	Female	63	Sigmoid
KRAS wild type	Male	50	Descending Colon
KRAS wild type	Male	60	Ascending Colon
KRAS wild type	Male	46	Sigmoid
KRAS wild type	Male	52	Sigmoid
KRAS wild type	Female	59	Sigmoid
KRAS wild type	Female	80	Ascending Colon
KRAS wild type + BRAF wild type	Female	54	Sigmoid
KRAS wild type + BRAF wild type	Female	38	Rectum
KRAS wild type + BRAF wild type	Female	61	Rectum
KRAS mutation, Codon 12 Arg	Female	53	Descending Colon
KRAS mutation, Codon 12 Val	Male	75	Descending Colon
KRAS mutation, Codon 12 Val	Male	80	Descending Colon
KRAS mutation, Codon 12 Asp	Male	76	Sigmoid
KRAS mutation, Codon 12 Val	Male	79	Sigmoid
KRAS mutation, Codon 13 Asp	Male	70	Sigmoid

DISCUSSION

Colorectal cancer (CRC) is the second leading cause of cancer death in men and women in the USA and is also the third most common neoplastic disease worldwide [5]. This disease is common in Iran [6]. The **Table 3** shows that the mean age in patients with CRC in different areas of Iran is between 50 and 60, and also in majority of regions, the percentage of men is more than women. Therefore, this report illustrated an increasing trend for CRC in men. Age range shows that the number ofyoung patientsisalso rising.

Table 3: Thecharacteristics ofpatients with colorectal cancer indifferent regionsof Iran

Reference	Place ofstudy	Number of Patients	Mean Age(year)	Age range(year)	Percentage of Men (%)
[6]	East	119	60.8	-	50.5
[7]	East	1001	55	40-88	47.7
[8]	Center	232	57.3	-	50.9
[9]	North	121	58.7	-	54.9
[10]	Northwest	52	54.1	-	-
[11]	Center	559	63	23-88	-
[12]	Northwest	546	55.2	-	56
[13]	South	792	59.4	18-93	52
[14]	Center	432	56	20-86	56.1
[15]	Center	442	53	-	49.8
[16]	North	9007	55.5	-	54
[17]	North	293	52.6	-	51.8
The present study	West	186	54.27	22-84	55.4
[18]	West	112	52.2	24-88	53.6
[19]	Center	218	53.3	15-87	64.2

The frequent anatomical locations for CRC are different. Two studies [8,18] reported that rectum and sigmoid colon and another study in Iran [19], reported that rectum and sigmoid had more frequent in patients. Also, a result of our study is near to resultsof thesestudies. Therefore, in Iran the left-side cancers are more prevalent in patients with CRC.

Three studies in Csenter of Iran [6,11,20] reported that the 5-year survival rate for patients with CRC is 55%, 68.3% and 47% respectively, but in our study is 75%. Therefore, these results show that mortality in patients with CRC in Western Iran is less than center and probably environmental conditions and lifestyle, including diet and exercise [2] effect on risk of CRC that these conditions in Center of Iran effect on more deaths of patients.Our studyshows thatthe 5-year survival rate in women is better than men andisstatistically significant ($P<0.05$), and a study in Iran [21] confirmed our result.

In CRC, BRAF and KRAS mutations are mutually exclusive, but both are independent prognostic factors for the disease [22].The association between KRAS mutations and response to EGFR inhibitors has been established in multiple studies; consequently, KRAS genotyping is recommended in all patients with metastatic CRC before any therapy that utilizes the EGFR-targeted monoclonal antibodies, cetuximab or panitumumab. Nevertheless, not all patients with KRAS wild-type tumors respond to EGFR-targeted therapies and the majority of the initially responsive patients experienced disease progression within 5 to 6 months [23].KRAS mutations in codons 12 and 13 are present in ∼40% of all CRCs but in our study, 6/186 or 3.2% of patients had KRAS mutations. Activating mutations in codons 61 and 146 of KRAS and in codons 12, 13, and 61 of NRAS also occur but are less frequent [24].

In our study, the OS for patients with KRAS wild-type was more than patients withKRAS mutations, but it was no statistically significant ($P>0.05$) and our results was similar to results of De Roock *et al.;*[25] and Payandeh *et al.;*[26]. Baskin *et al.;*[27] reported that location of tumor (colon or rectosigmoid) for patients with KRAS wild-type or KRAS mutations was more in colon and KRAS wild-type in all patients was more in males. In our study, KRAS wild-type or KRAS mutations are more in patients with rectum or left colon (21/24 patients (87.5%)) and also KRAS wild-type is more in males. Also, in this study, of 18 patients with KRAS wild-type, 3 patients (16.7%) had BRAF wild-type, too that all of them were female.

CONCLUSIONS

First of all, CRC in Iran is common. Second, CRC is more in males and in age of above 60 years in Iran. Third, there is not the relationship between sex and the age in patients. Fourth, KRAS wild-type is more than KRAS mutations in patients in western Iran and location of tumor in the patients is more in the rectum or left colon. At last, the OS rate in females is significantly higher than males ($P<0.05$).

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