ORIGINAL ARTICLE

Prevalence and Predictive Value of RAS Mutations in Metastatic Colorectal Cancer at Hiwa Cancer Hospital, in Sulaimani Iraq

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ABSTRACT

Background and objective: Colorectal cancer (CRC) is the second most fatal and the third most common malignant tumor in the world. Mutation in NRAS and KRAS genotypes has been reported in this type of cancer. The present study was conducted to examine the prevalence of these two genotypes in CRC and their association with clinical outcomes of patients with advanced CRC.

Materials and methods: The present study was a prospective one conducted on 81 stage IV metastatic colorectal cancer patients who were cured with and without chemotherapy at Hiwa Cancer Hospital in Sulaimani, Iraq from January 2016 to December 2019. KRAS and NRAS mutations were tested in the patients on formalin-fixed paraffin-embedded tissue. The collected data were analyzed through Statistical Package for Social Sciences (version 23.0).

Results: The patients' mean age was 53.5 years. CRC was more prevalent among males. KRAS mutant alone, and KRAS and NRAS mutants were respectively seen in 30.9% and 6.1% of the patients. Prevalence of stage IV CRC was 30.5%, and RAS genotype prevalence was 37%. KRAS and NRAS status had no significant association with the patients' characteristics like sex, age, primary site, tumor grade, or smoking (p-value>0.05). Although females had a better 1-year survival outcome, it did not have a significant association with sex (p-value=0.49). One-year survival was significantly better in patients with chemotherapy with treatment (p-value=0.01) and grade I CRC (p-value=0.05). Overall survival was not significantly different between males and females, different ages, or different tumor sites (p-value>0.05), while it was significantly better in grade I CRC patients (p-value=0.04) and those who had chemotherapy with treatment (p-value=0.01). One-year survival and overall survival were not significantly different between patients undergone chemotherapy with Cetuximab and Bevacizumab (p-value>0.05).

Conclusion: The prevalence of KRAS mutant alone, and KRAS and NRAS mutants were respectively 30.9% and 6.1%. Factors like sex, age, tumor initial site, tumor grade, and smoking have no significant association with KRAS and NRAS genotype status. Females had better survival outcomes than males. Determination of KRAS and NRAS genotype in metastatic CRC is required for choosing the most appropriate therapy.

Keywords: colorectal cancer (CRC), KRAS genotype, NRAS genotype, clinical outcomes, survival

INTRODUCTION

As the second most fatal cancer, colorectal cancer (CRC) has been referred to as the third most common malignant tumor all over the world. According to 2018 reports, there were 1.8 million new cases of CRC, among who 881,000 died. This statistic formed almost 10% of new cases of cancer and deaths around the world. It has been estimated that there will be 2.5 million new cases in 2035 [1].

CRC patients usually experience cancer-related mortality mainly due to metastases [2]. The most common sites that colon cancer metastasizes include the peritoneum, the lung, the liver, and the regional lymph nodes [2,3]. At initial diagnosis, about 25% of CRC patients have distant metastatic disease, and half of them will develop metastatic disease later on. Suffering from an untreatable disease has been reported in most patients with metastatic colorectal cancer (mCRC) [2]. The treatment of mCRC is one of the most astonishing successes over last decades. Among the successful treatments, targeted cancer therapy is a potent strategy for treating patients who are chosen based on their molecular characteristics [4].

As RAS oncogenes, the NRAS and KRAS encode a family of GTP-regulated switches that undergo recurrent mutation in human cancer. By preventing hydrolysis of GTP, activating mutations renders the RAS protein in the GTP-bound activated form, resulting in preventing transition to the GDP-bound inactive state. It has been proved that RAS mutations happen at conserved hotspots, while oncogenic mutations at codons 12, 13, 61, 117, and 146 [5]. Due to the activation of the RAS proteins, cells undergo pleiotropic impacts like cellular differentiation, survival, and proliferation. At present, it is not clear how downstream and signaling effectors caused by different activated RAS isoforms vary [6].

In CRC, the KRAS gene is where RAS mutations mainly happen. Moreover, an activating KRAS mutation is included in 45% of mCRC [7]. In addition, 2% to 7% of mCRC cases undergo NRAS mutations [8]. As a part of regular RAS testing and before epidermal growth factor receptor (EGFR) inhibitor therapy, the clinical treatment of mCRC is normally associated with identifying NRAS mutations. As proved by clinical evidence, the response of NRAS mutation to anti-EGFR therapy and the behavior of

these tumors compared to KRAS-mutant mCRC are still unknown [8].

Research has proved association between NRAS mutations and left-sided primary tumors and female gender, and between KRAS mutations and right-sided colon tumors, implying separate biology for NRAS- and KRAS-mutant molecular subsets of metastatic colorectal cancer [6]. RAS mutations have been referred to as helpful indicators for prediction of the responses to anti‑EGFR monoclonal antibodies in mCRC [9,10]. In most countries in the world, KRAS mutation have been reported to vary between 20 and 50 percent, while the occurrence of NRAS mutations is rare, ranging from 3% to 5% of CRC [11]. However, it is not yet clear how the frequency of NRAS mutations are related with molecular, pathologic, and clinical features [12].

Recent clinical studies have examined all NRAS- or RAS-mutant CRC; however, NRAS-mutant cases are rare, the results have been limited. Worse survival in mCRC has been observed in KRAS and all RAS mutations [13]. Worse outcomes and increased risk of recurrences in the lungs have also been reported in KRAS and all RAS mutations after hepatectomy [14,15].

In this regard, the present study was aimed at examining the incidence and clinical outcomes of KRAS and NRAS mutations in metastatic colorectal cancer patients admitted to Hiwa Cancer Hospital in Sulaimani, Iraq from January 2016 to December 2019.

MATERIALS AND METHODS

Study design and setting: The present prospective and retrospective study was carried out on the cohort of patients with metastatic colorectal cancer (stage IV), who were treated using chemotherapy with or without targeted therapy in Hiwa Cancer Hospital in Sulaimani, Iraq from January 2016 to December 2019.

Patients: A total of 81 patients were included in the present study, who were chosen based of the following inclusion criteria:

- Patients with stage IV colon and rectal cancer;
- Patients with previous localized colorectal cancer represented with metastatic recurrence;
- Adenocarcinoma confirmed on histopathologic examination of the tissue biopsy; and
- KRAS and NRAS genotype test performed on the tissue biopsy.

Data collection: Required data were reviewed on the clinical portal system of Hiwa Cancer Hospital during the mentioned period. This clinical database system records almost all the clinical data and investigations of patients referred to the hospital, including demographics, histopathology reports, imaging reports, diagnosis, MDT recommendations, treatment, and follow-up. For some patients who lacked full information records in the database, their data were obtained from the referring hospital, laboratory, clinic, or directly from the patients' documents.

Procedures: The targeted therapies included Cetuximab and Panitumumab which are Epidermal Growth Factor Receptor (EGFR) inhibitors used to treat patients with metastatic colorectal cancer with wild KRAS and NRAS genotypes. Based on the availability of the drug in Hiwa Cancer Hospital, Cetuximab was the selected drug in this study. Testing for KRAS and NRAS mutations were performed on formalin-fixed paraffin-embedded tissue in certified laboratories containing all required equipment.

Statistical analysis: The data were obtained over a period of 22 months, from July 2018 to April 2020, and were organized on a Microsoft Excel worksheet using Microsoft Excel 2016 software program. The organized clinical and demographic data were then analyzed using the Statistical Package for Social Sciences (SPSS) software program version 23.0. Statistical analysis was performed to describe the one-year both wild and mutant RAS genotype groups. Furthermore, survival outcomes were analyzed for the group of patients with wild RAS genotype, who have received Cetuximab. Overall survival was defined by the time from the initial diagnosis to death from any cause or censored on the date last seen alive. An independent sample t-test was used to compare the mean ages of both groups. A p-value less than 0.05 was considered to be statistically significant.

RESULTS

The study consisted of a total of 81 patients with a mean age of 53.5± 17 years. The male to female ratio was 1.3:1. Considering the primary site of the tumor, 31 (38%) patients had primary rectal cancer, and the remaining 50 patients (62%) had their tumor originating from the colon (16 patients in the right colon and 34 in the left colon). According to laboratory test for genotype of the tumors, 51 patients (63%) had wild-type KRAS and NRAS genotype and 30 patients (37%) harbored RAS mutation. Of the 30 patients with mutation in their RAS genes, 25 patients revealed KRAS mutation alone, and only five patients (6.2%) showed mutations in both KRAS and NRAS genes.

Table 1. Characteristics of patients and their tumors

Characteristics	Number (%)
Number of Patients	81
Age (year)	
Median	55
Range	20-87
Mean	53.5
Sex	
Male	46 (56.8)
Female	35 (43.2)
Male/Female ratio	1.3:1
Site of tumor	
Rectum	31 (38.3)
Left Colon	34 (42.0)
Right Colon	16 (19.8)
KRAS and NRAS genotype	
Wild-Genotype	51 (63.0)
KRAS Mutant Only	25 (30.9)
KRAS and NRAS Mutant	5 (6.1)
Grade of Tumor	
I	12 (14.8)
II	57 (70.4)
III	12 (14.8)
Smoking (current or x-smoker)	
Yes	31 (38.3)
No	50 (61.7)

Furthermore, the histopathological result of the tissue samples showed that majority of the tumors [57 (70%)] were grade II disease, and the remainder 24 patients were evenly distributed between grade I and III tumors (15%

grade I and 15% grade III). Approximately one third of the patients (38%) were either current or x-smoker, and only 5 out of 81 patients (6%) were ex-alcohol drinker (See Table 1).

The total number of 702 patients with colorectal cancer patients were admitted to Hiwa Cancer hospital in Sulaimani, Iraq from 2016 to 2019, of whom 214 were stage IV colorectal cancer. As shown by the results, during the above-mentioned period, the prevalence of stage IV colorectal cancer in Sulaimani, Iraq was 30.5% and the frequency of KRAS and NRAS mutation among stage IV colorectal cancer patients was 37% (See Table 2)

The two subgroups were not significantly different (p-value>0.05). The patient and disease factors, such as age, sex, smoking, primary site of the tumor, and grade of differentiation and, are nearly fairly distributed between the study subgroups in order to minimize their undesired influence on the results of the study and minimize error (See Table 3).

Table 2. Prevalence of stage IV colorectal cancer and frequency of KRAS and NRAS mutation among the study sample

Patients	Number (From 2016 to 2019)	Prevalence (Frequency)
Colorectal Cancer Patients	702	-------
Stage IV Colorectal Cancer Patients	214	30.5%
Study Sample	81	-------
Mutant RAS Genotype Among Study Sample	30	37%

Table 3. Distribution of KRAS and NRAS genotype status among study patients

Characteristics		K-RAS & N-RAS Status		Total (%)	P-value
		Mutant (%)	Wild (%)		
Sex	Male	16 (34.8)	30 (65.2)	46 (100.0)	0.63
	Female	14 (40.0)	21 (60.0)	35 (100.0)	
Age	< 40	9 (40.9)	13 (59.1)	22 (100.0)	0.78
	40 - 60	11 (39.3)	17 (60.7)	28 (100.0)	
	> 60	10 (32.3)	21 (67.7)	31 (100.0)	
Primary Site	Colon	17 (34.0)	33 (66.0)	50 (100.0)	0.47
	Rectum	13 (41.9)	18 (58.1)	31 (100.0)	
Grade	1.0	4 (33.3)	8 (66.7)	12 (100.0)	0.94
	2.0	21 (36.8)	36 (63.2)	57 (100.0)	
	3.0	5 (41.7)	7 (58.3)	12 (100.0)	
Smoking (Current or ex-smoker)	Yes	10 (32.3)	21 (67.7)	31 (100.0)	0.48
	No	20 (40.0)	30 (60.0)	50 (100.0)	
Total		30 (37.0)	51 (63.0)	81 (100.0)	

Out of the 81 patients, the genotype of 30 patients showed mutation in KRAS or both KRAS and NRAS genes who then received chemotherapy with bevacizumab (an anti-VGFR antibody), except two of them who refused to receive treatment. The remaining 51 patients who carried no mutation in KRAS or NRAS genes received either chemotherapy with bevacizumab (28/51 patients), chemotherapy with Cetuximab (17/51 patients), or chemotherapy alone (4/51 patients) (See Figure 1).

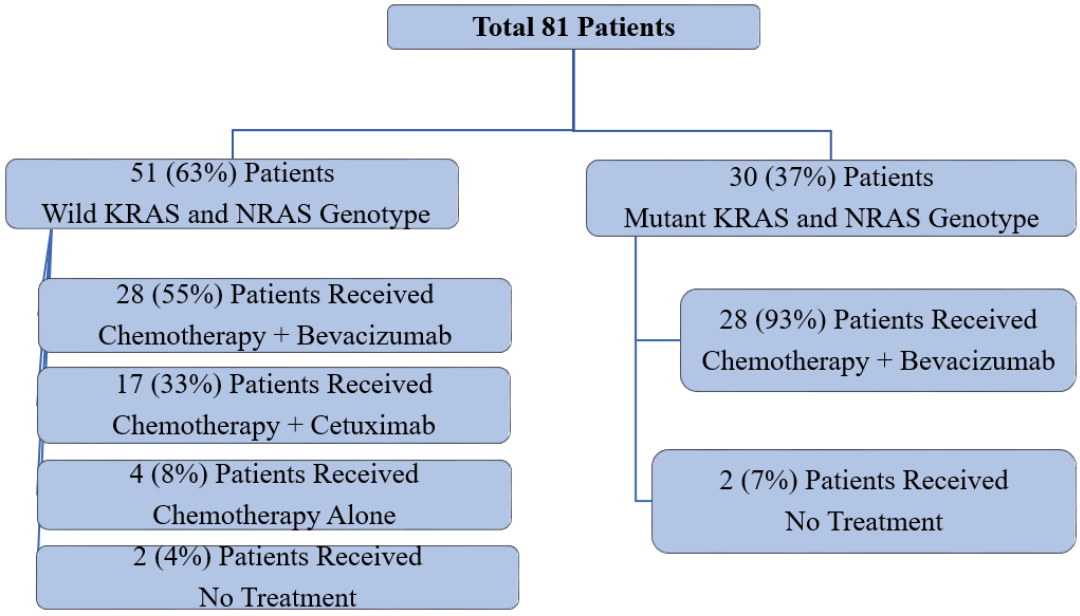

Figure 1. Types of treatment regimens given to study subjects based on the KRAS and NRAS genotype of their tumors

Clinical outcomes, mainly 1-year and overall survival outcomes, of the study subjects were analyzed in relation to several patient and tumor factors including sex, age, smoking, site of primary tumor, KRAS and NRAS genotype

status, and type of targeted therapy prescribed. Only 5 patients were x-drinker of alcohol. In general, females tend to have better 1-year survival outcomes compared to males and the Hazard Ratio is (HR = 1.3), although it is statistically not significant (P-value = 0.49) (See Figure 2).

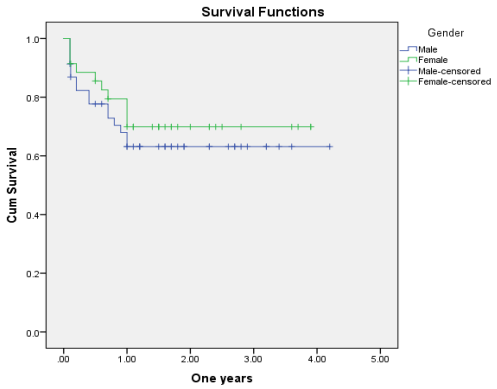

Figure 2. One-year survival outcomes of males and females

Considering primary site of the tumor, analyzed data showed no significant difference in the 1-year survival outcome colon and rectum (P-value = 0.41). The result is the same for right and left colon (See Figure 3).

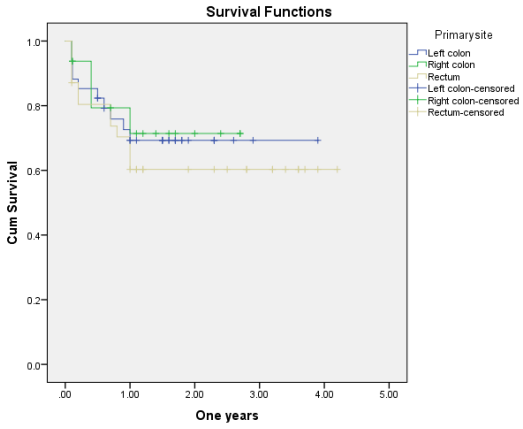

Figure 3. One-year survival outcomes primary right colon, left colon and rectum tumors

Patients who received chemotherapy with Cetuximab, the targeted therapy of interest in this study based on KRAS and NRAS genotype, had best 1-year survival outcome among all other subgroups who received chemotherapy with Bevacizumab, chemotherapy alone or no treatment (i.e. best supportive care). This positive impact of Cetuximab on 1-year survival outcome of study subjects is statistically significant (P-value = 0.01) (See Figure 4).

In this study, 1-year survival outcomes for grade I, II, and III colorectal cancer patients are 90%, 65% and 50%, respectively. This indicates that colorectal cancer patients whose tumor show grade I differentiation on histopathological examination carry a statistically significant favorable 1-year survival outcome compared to those with grade II or III disease (P-value = 0.05) (See Figure 5).

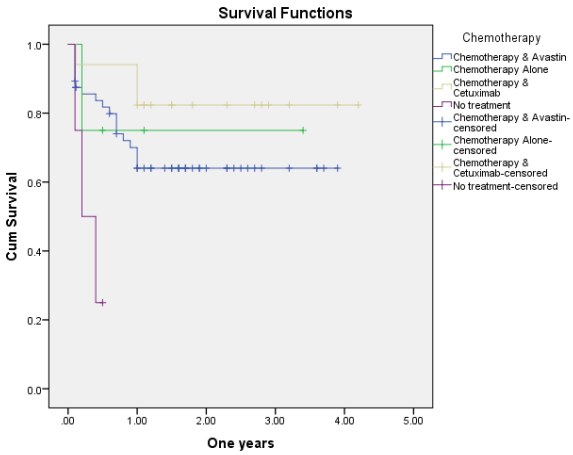

Figure 4. One-year survival outcomes of the study subgroups receiving different treatment protocols and targeted therapies

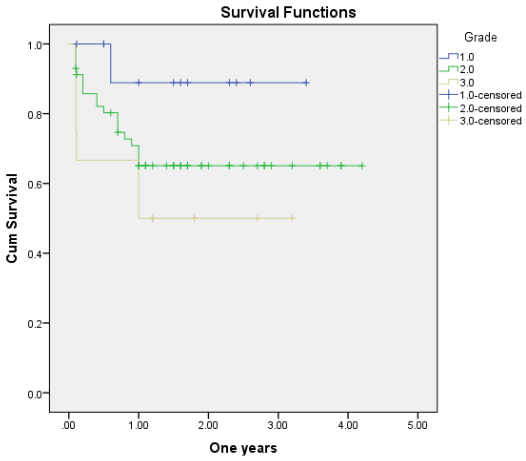

Figure 5. Impact of grade of the primary colorectal cancer on 1-year survival outcome

In addition to that, there was a statistically significant difference in the overall survival of patients with grade I colorectal cancer compared to grade II and III tumors (P-value = 0.04) (See Figure 6).

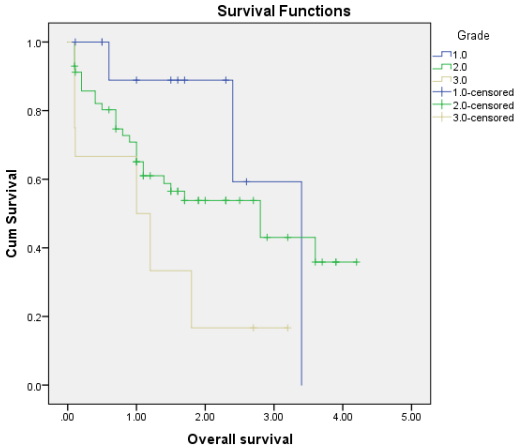

Figure 6. Impact of grade of the primary colorectal cancer on the overall survival outcome

Furthermore, the overall survival outcome of patients who received chemotherapy with Cetuximab stayed better than all other treatment arms including chemotherapy with Bevacizumab and chemotherapy alone. This favorable effect of the anti-EGFR targeted therapy on the overall survival was statistically significant (P-value = 0.01) (See Figure 7).

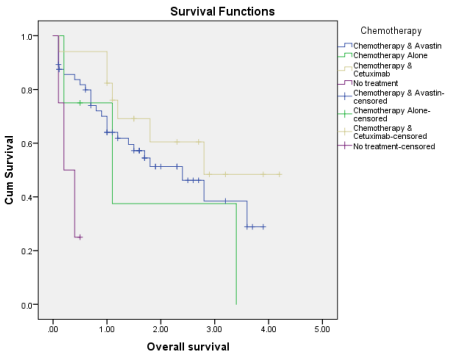

Figure 7. Overall survival outcome of study subjects receiving different treatment protocols

Among the study sample, laboratory result of 51 (out of 81) patients with stage IV colorectal cancer showed wild KRAS and NRAS genotype. Seventeen patients received chemotherapy with Cetuximab (an anti-EGFR used as targeted therapy in this study) and 28 patients received chemotherapy with Bevacizumab (an anti-VGFR used in advanced colorectal cancer). The remaining 6 patients received either chemotherapy alone or no treatment (See Table 4).

Table 4. Treatment regimens and targeted therapies used in the wild KRAS and NRAS genotype subgroups

Study Sample	Number (%)
Wild KRAS and NRAS Genotype Subgroup	51 / 81 (62.9%)
Chemotherapy + Cetuximab Arm	17 (33.3%)
Chemotherapy + Bevacizumab Arm	28 (54.9%)
Others	6 (11.7%)

One-year survival outcomes demonstrated that 83% of patients in the Cetuximab arm survived after 1 year, whereas 65% of study subjects in the Bevacizumab arm survived during the above period of time. Although this difference was statistically not significant (P-value = 0.19), there is a clear separation of the two groups on the Kaplan-Meier survival plot (See Figure 8).

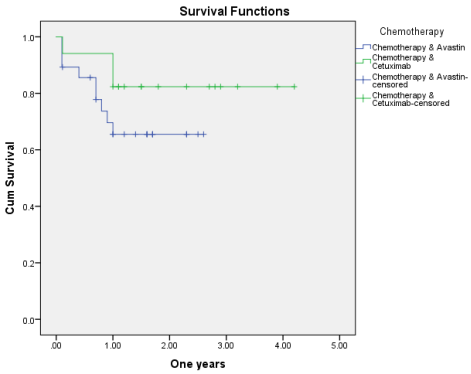

Figure 8. One-year survival outcome of the wild KRAS and NRAS genotype subgroup in relation to type of chemotherapy and targeted therapy

Among the wild genotype subgroup, the overall survival outcomes for both Cetuximab and Bevacizumab arms is approximately 50% (P-value = 0.32). Kapan-Meier graph shows that both groups keep separated continuously and there is no crossing point between both arms (See Figure 9).

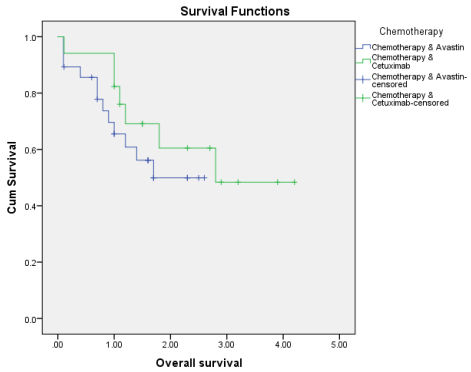

Figure 9. Overall survival outcome of the wild KRAS and NRAS genotype subgroup in relation to type of chemotherapy and targeted therapy

DISCUSSION

The present study consisted of 81 patients that were investigated to specify the prevalence rate of KRAS and NRAS mutation in advanced colorectal cancer and their association with clinical outcomes. The results demonstrated that CRC patients' mean age was 53.5. Also, more than half of the patients were men, and the three particular sites of the tumors were the rectum, the left colon, and the right colon. The rate of tumor incidence in the left colon, with a percentage of 42%, was higher in comparison with two other sites. In this regard, in their study, Golfam et al referred to the rectum and sigmoid as the most common sites for tumors. They also stated that men and women are not significantly different in terms of the tumor site. In addition, they reported no significant relationship between the patients' age and the pathologic pattern. Finally, they observed that pathologic patterns are the same in both young and old patients [16].

According to the provisional clinical reports by the American Society of Clinical Oncology, KRAS mutations should be tested in the tumors of the mCRC patients who candidates for anti-EGFR antibody therapy. In the present study, more than two-thirds of patients had wild KRAS and NRAS genotypes. After the right KRAS and NRAS genotype is detected, the most proper therapy can be adopted. In this regard, Saridaki et al reported that therapy is more likely to be effective for patients with a wild KRAS genotype [17]. Grade II tumors, which are defined as moderately differentiated, were observed in over 70% of the cases. Furthermore, about a bit more than one-third of the cases had mutant RAS genotype.

The results of the present study revealed that there was a significant association between 1-year survival outcomes and grades of primary colorectal cancer, such that higher grades of colorectal cancer resulted in lower than 1-year survival outcomes. Similarly, the results of the study conducted by Zacharakis et al indicated that treatment of CRC patients with cetuximab could bring about more proper outcomes in patients with grades 1, 2,

and 3 CRC [18]. Moreover, a significant association was observed between overall survival outcome and the grade of primary CRC, implying that better survival outcomes can be obtained in patients with lower grades of cancer.

In this study, the female patients tended to have better 1-year survival compared to the males. Although this clinical outcome is quite clear on the Kaplan-Meier graph (Figure 2), it is not statistically significant. According to international data on the effect of gender on survival outcomes of colorectal cancer, female sex has generally been stated as a favorable prognostic factor, which agrees with the results of the current study, though data are limited and inconclusive [19].

In a prospective study conducted by Cheung et al., 33,345 patients participating in the ACCENT database of randomized trials were recruited, and the results revealed a significant but very modest survival advantage for females with early-stage disease that persisted across all ages, stages, and types of adjuvant therapy. They have concluded that sex was not a predictive factor for treatment efficacy and the decision regarding chemotherapy regimens should not be based on this parameter [20].

Investigating the effect of some factors of sex, age, the primary site of the tumor, tumor grade, and smoking showed that these factors could not significantly affect the distribution of KRAS and NRAS genotype status among the studied patients. Similar to this finding, the results of some recent studies indicated no significant association between gender and KRAS and NRAS mutations [21,22]. The data from the present study demonstrated that about two-thirds of the patients were detected with wild KRAS and NRAS genotype, and half of them received chemotherapy in combination with bevacizumab as treatment regimens. In this regard, different results have been reported for administrating a combination of chemotherapy and bevacizumab therapies on patients with wild KRAS and NRAS genotype. Moreover, Bencsikova et al showed that KRAS mutations do not have any impact on the results of first-line bevacizumab combinations with chemotherapy [23]. Also, Bruera et al have reported bevacizumab therapy can lead to significantly shorter survival of the patients with KRAS and NRAS mutation and a possible effect of the KRAS genotype on angiogenesis [24].

Moreover, around one-third of the patients with wild KRAS and NRAS genotype received a combination of chemotherapy with cetuximab as therapeutic options. According to the results, patients who received a combination of chemotherapy with cetuximab-censored, chemotherapy alone-censored, and chemotherapy with Avastin-censored experienced a higher 1-year survival outcome compared to those who did not receive treatment censored. In line with the present outcomes, Bokemeyer et al indicated that cetuximab monotherapy led to the primary response rates of about 10% in patients with heavily pretreated mCRC, while tumors without mutations in codon 12 or 13 of the KRAS gene gradually responded in 13–17% of cases, and only 0–1.2% of the KRAS mutant tumors did [25]. Moreover, it has been discovered that mutant KRAS is accompanied by resistance to anti-EGFR monoclonal antibodies, implying that tumors of all patients with mCRC are now profiled for seven KRAS codon 12 and 13 mutations prior to receiving panitumumab or cetuximab [26,27]. The results of a similar study indicated the ineffectiveness of cetuximab in patients with BRAF mutant mCRC, recommended in smaller series. However, is looks quite unlikely because the absolute advantage from cetuximab treatment is very small for patients with chemotherapy-refractory mCRC with a BRAF-mutant tumor, in comparison with the BRAF wild-type population [28].

The current study used chemotherapeutic combinations that proved to be effective in metastatic colorectal cancer based on clinical trials. The study subjects received one of mFOLFOX6, CAPEOX, and FOLFIRI chemotherapeutic regimens with or without targeted therapy based on the internationally approved therapy protocols [29-33]. Only a few patients with poor performance status that were unfit for intensive therapy received either single-agent chemotherapy or best supportive care [34]. The 1-year and overall survival outcomes of all patients in different treatment arms were analyzed. In general, study results demonstrated that patients who received chemotherapy with cetuximab (the targeted therapy of interest used in the current study based on KRAS and NRAS genotype of the study subjects) had significantly better 1-year and overall survivals compared to all other subgroups who received chemotherapy with Bevacizumab, chemotherapy alone or no systemic treatment (i.e. best supportive care). These clinical outcomes of our study are in line with most internationally conducted studies and clinical trials concerning the effect of anti-EGFR therapies (cetuximab and panitumumab) on the survival of patients with metastatic colorectal cancer [35-37]. Thus, based on the above results, among most systemic regimens, cetuximab combined with chemotherapy can be regarded as the treatment of choice for eligible (wild KRAS and NRAS genotype), metastatic colorectal cancer patients.

The results of the present study indicated that the survival outcomes of females are better than males. In line with this finding, Yang et al referred to gender as an important criterion that affects survival results among patients with CRC [38]. Another study revealed that men had similar survival rates to women who had never been pregnant or those with no children, but significantly different to women with children one or more [39].

Based on the analysis in this study, there was no significant difference in the 1-year survival outcome colon and rectum by considering the primary site of the tumor. Brouwer et al carried out a similar study and showed that the right colon had worse 1-year relative survival of 40% in comparison with 51% for left colon and 54% for rectal cancer [40]. In addition, compared with left colon and rectal cancer, the relative survival rate was significantly worse for the right colon. However, they stated that relative excess risks for death are also shown after correction for radiotherapy, metastasectomy, chemotherapy, primary tumor resection, diagnosis period, morphology, age, gender, and primary tumor location [40]. Moreover, the staging system taking into account the site of metastasis might lead to better treatment of risk stratification and a more accurate prediction of survival in patients with colon cancer [41].

CONCLUSION

Men were found to suffer from colorectal cancer more than women, and their mean age varied around 53 years. However, the survival outcome of females was better than males. The most appropriate therapy can be chosen after the KRAS and NRAS genotype is determined and the stage of colorectal cancer is found. Among all systemic therapy regimens used in the present study, chemotherapy with Cetuximab provided the best 1-year and overall survival outcomes compared to other regimens. Furthermore, patients with wild KRAS and NRAS genotype carried a better prognosis when treated with chemotherapy with Cetuximab compared to chemotherapy with bevacizumab, and hence the above mutations can be regarded as predictive factors for using Cetuximab in such cancers. Additionally, Cetuximab is safer and less toxic than bevacizumab in the metastatic colorectal cancer setting, though bevacizumab is reasonably less costly compared to Cetuximab. There was no Cetuximab/bevacizumab-related death reported in this study. These tumors have distinct biology, both in development sites and computation patterns; therefore, further understanding of their biology is needed to better target these tumors.

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