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Our data on detailing metastasis localization and subtype characteristics in metastatic colorectal cancer patients treated with Bevacizumab

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ABSTRACT

Aims: Our aim in this study was to determine the relationship between metastasis types and mutation subtypes in patients who were followed up in our center and received bevacizumab treatment, to determine the survival rates according to metastasis types, and to contribute to the literature on this subject.

Methods: In our study, we retrospectively evaluated 42 consecutive metastatic colorectal cancer patients who were admitted to our hospital and diagnosed with colorectal cancer, thorax-abdominal CT scans were performed in our clinic to detect possible metastases, and the presence of metastases in one or more localizations was detected and treated with Bevacizumab.

Results: The majority of colorectal cancers included in our study had the histopathological subtype of adenocarcinoma (90.5%). Genetic analyses revealed that 47.6% (20 patients) had mutant KRAS gene types, while 52.4% (22 patients) had wild type. The distribution of metastases was as follows; 31 (73.8%) cases with liver involvement, 12 (28.6%) with peritoneal involvement and 24 (57.1%) with lung involvement. In our study, median overall survival was 19 months and median disease-free survival was 7 months.

Conclusion: The results of studies to date will be useful to help predict prognosis and to select appropriate regimens for treatment. We aimed to contribute to this process by presenting our own data in our own study. However, the true role of RAS genes as prognostic markers continues to be questioned, and multicenter studies are needed on the predictive and prognostic factors of colorectal cancers.

Keywords: Colorectal cancer, kras, metastasis, prognosis

INTRODUCTION

Colorectal cancer is the third most common cancer in the world, and although mortality rates have decreased in recent years, it is still a major cause of morbidity and mortality in both men and women worldwide. Although colorectal cancers are known to be cancers of advanced age, the incidence of early-onset colorectal cancer diagnosed in patients younger than 50 years of age is increasing worldwide and is becoming a cancer affecting a younger patient population. In addition, due to the absence of obvious symptoms in the early stages of colorectal cancer, distant metastasis rates accompanying the primary tumor diagnosis are high, and fifty percent of metastatic patients die within 5 years of diagnosis, usually as a result of metastatic disease.[1-3] This reality is another source of concern for colorectal cancers and has led to an increased interest in the epidemiology of colorectal cancers and the determinants of the treatment process. Today, traditional prognostic factors have been replaced by new outcome predictors, including those defined according to the molecular origin of the primary tumor. In particular, RAS or BRAF mutation status and biomarkers have recently been introduced into clinical scoring systems and are increasingly becoming an integral part of oncosurgical treatment algorithms.[4,5]

METHODS

The study was carried out with the permission of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Clinical Researches Ethics Committee (Date: 21.12.2022, Decision No: 2022-12/5). All procedures were performed in accordance with ethical rules and the principles of the Declaration of Helsinki.

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Forty-two consecutive patients who were diagnosed with metastatic colorectal cancer in our clinic and underwent thorax-abdominal CT scanning to detect possible metastases were evaluated retrospectively. After the detection of one or more metastases, adjuvant chemotherapy followed by bevacizumab treatment was administered. The patients included in our study received fluorouracil, leucovorin, and oxaliplatin (FOLFOX), fluorouracil, leucovorin, irinotecan (FOLFIRI), and fluorouracil, leucovorin (FUFA) as adjuvant chemotherapy agents. FOLFOX was administered to 67% (28), FOLFIRI to 23%, FOLFOX+FUFA to 5% (2), and FUFA to 5% of the patients. The maximum treatment regimen was six cycles, and the minimum treatment regimen was one cycle. In our patient population, bevacizumab was administered as monoclanal anicor for etastatic colorectal cancers. Bevacizumab was given alone in ten patients; bevacizumab + FOLFOX combination in ten patients; bevacizumab + FOLFIRI combination in 11 patients; bevacizumab + FUFA combination in 9 patients; and bevacizumab was accompanied by Xelox and Capecitabine in only one patient. Bevacizumab, the component of complementary therapy, was administered for a maximum of 30 cycles and a minimum of 5 cycles.

Statistical Analysis

In this study, patient data collected was analyzed using IBM Statistical Package for the Social Sciences (SPSS 23.0-IBM, NY, USA) and MedCalc statistical software version 12.7.0.0 (MedCalc Software, Ostend, Belgium) package programs. Frequency and percentage were provided as descriptive statistics for categorical variables, while median, minimum, and maximum values were used to describe continuous variables. Kaplan-Meier curves were plotted for overall survival and disease-free survival, and the Log-rank test was used to compare the groups. Results were considered statistically significant when the p-value was less than 0.05.

RESULTS

A total of 42 patients, 26 (61.9%) males and 16 (38.1%) females, were included in the study. The age of the patients ranged between 21 and 81 years with a median age of 59 years. Tumor localization was 50% (21 patients) in the colon, 45.2% (19 patients) in the rectum and 4.8% (2 patients) in the colorectal region. The majority of tumor subtypes were adenocarcinomas (90.5%). When the KRAS gene types of the patients were analyzed, 47.6% (20 patients) had mutant type and 52.4% (22 patients) had wild type. There were 31 (73.8%) with liver involvement, 12 (28.6%) with peritoneal involvement, and 24 (57.1%) with lung involvement. The overall median survival was 19 months, and the median disease-free survival was 7 months. In our study, we also looked at the relationship between metastasis foci and survival. In peritoneal metastases, we found that the median overall survival was 12 months in patients with peritoneal involvement and 23 months in patients without peritoneal involvement, and there was a statistically significant difference between these two groups ($p=0.041<0.05$). In our disease-free survival analysis, the median progression-free survival of patients with and without peritoneal involvement was equal at 7 months, and no statistically significant difference was observed ($p<0.05$) **Figure 1**. When we looked at patients with liver involvement as another important metastasis focus, we found that the median overall survival was 22 months in patients with liver involvement and 15 months in patients without liver involvement, and there was no statistically significant difference between them ($p=0.399>0.05$) **Figure 2**. The median progression-free survival was 9 months in patients with liver involvement and 7 months in patients without liver involvement, and there was no statistically significant difference between them ($p=0.396>0.05$). When we looked at survival according to mutations, survival was 15 months in the KRAS mutant type and 23 months in the wild type. However, progression-free survival was the same in both groups and was 7 months. However, we did not find a statistically significant difference between the two genes in terms of overall survival ($p=0.438>0.05$). In terms of disease-free survival, the median progression times of both mutant and wild-type were equal at 7 months, and no statistically significant difference was observed between them ($p<0.05$). In our study, we went one step further and looked at the survival relationship between the KRAS mutation and metastasis localization. In terms of overall survival, the median survival was 13 months for mutants and those with liver involvement and 28 months for those without, while it was 16 months for wild-types and those with liver involvement and 18 months for those without. However, no statistically significant difference was observed between them ($p=0.533>0.05$). In terms of disease-free survival, the median progression time was 4 months for the mutant type and those with liver involvement and 7 months for those without, while it was 2 months for the wild type and those with liver involvement and 7 months for those without, and no statistically significant difference was observed between them ($p: 0.405>0.05$). Similarly, when the presence of peritoneal involvement was analyzed according to KRAS gene types, the median overall survival was found to be 18 months in patients with mutant type and peritoneal involvement and 28 months in patients without peritoneal involvement. The median survival was 11 months in patients with wild-type peritoneal involvement and 18 months in patients without peritoneal involvement. However, no statistically significant difference was observed between the groups ($p<0.05$). In disease-free

survival, the median progression-free survival was 7 months in patients with mutant gene and peritoneal involvement and 9 months in those without, while the median progression-free survival was 9 months in patients with wild-type gene and peritoneal involvement and 7 months in those without, and no statistically significant difference was observed (p:0.748>0.05) **Figure 3**. In terms of overall survival, the median survival time for patients with mutant type and lung involvement was 12 months and 28 months for those without, while the median survival time for patients with wild type and lung involvement was 13 months and 18 months for those without. There was no statistically significant difference between the groups (p=0.100>0.05). In disease-free survival, the median progression time was 7 months for the wild type and those with lung involvement and 8 months for those without lung involvement. The median time to progression was 7 months for mutants and those with lung involvement and could not be calculated for those without lung involvement because the progression rate did not reach 50%. No statistically significant difference was observed (p=0.804>0.05) **Figure 4**.

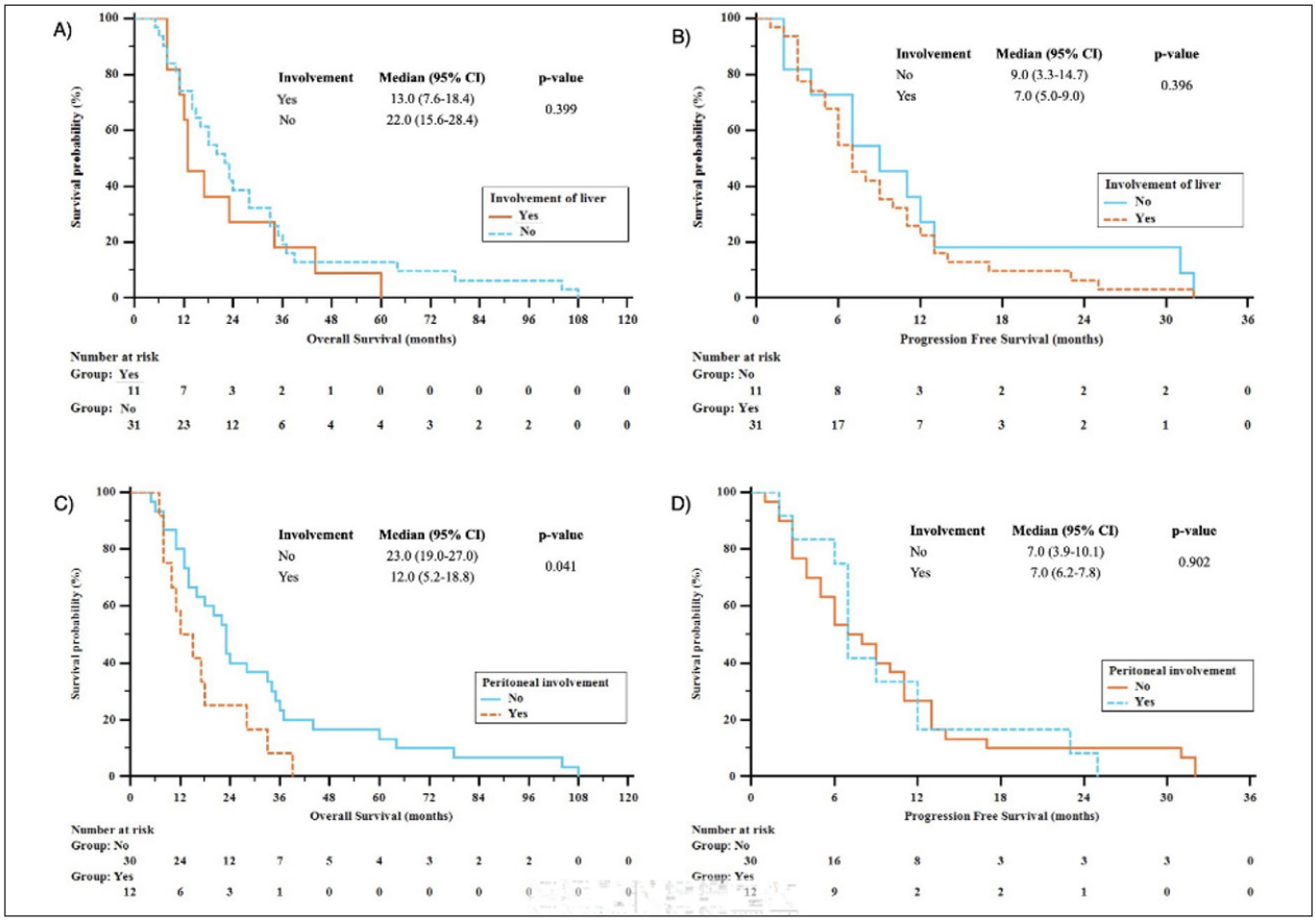

Figure 1. Liver involvement and peritoneal involvement

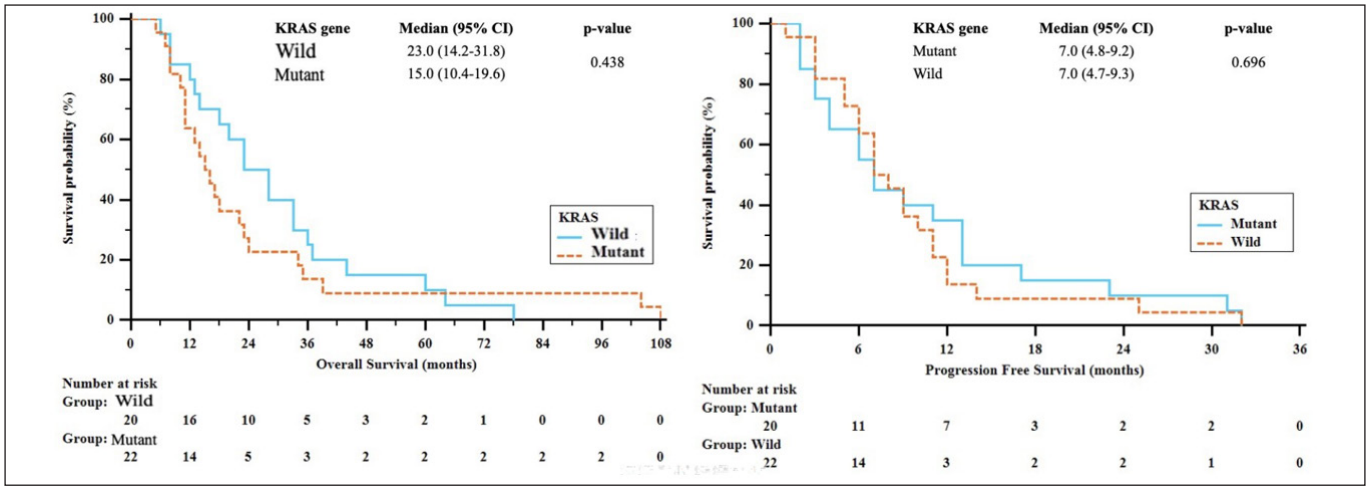

Figure 2. KRAS gene

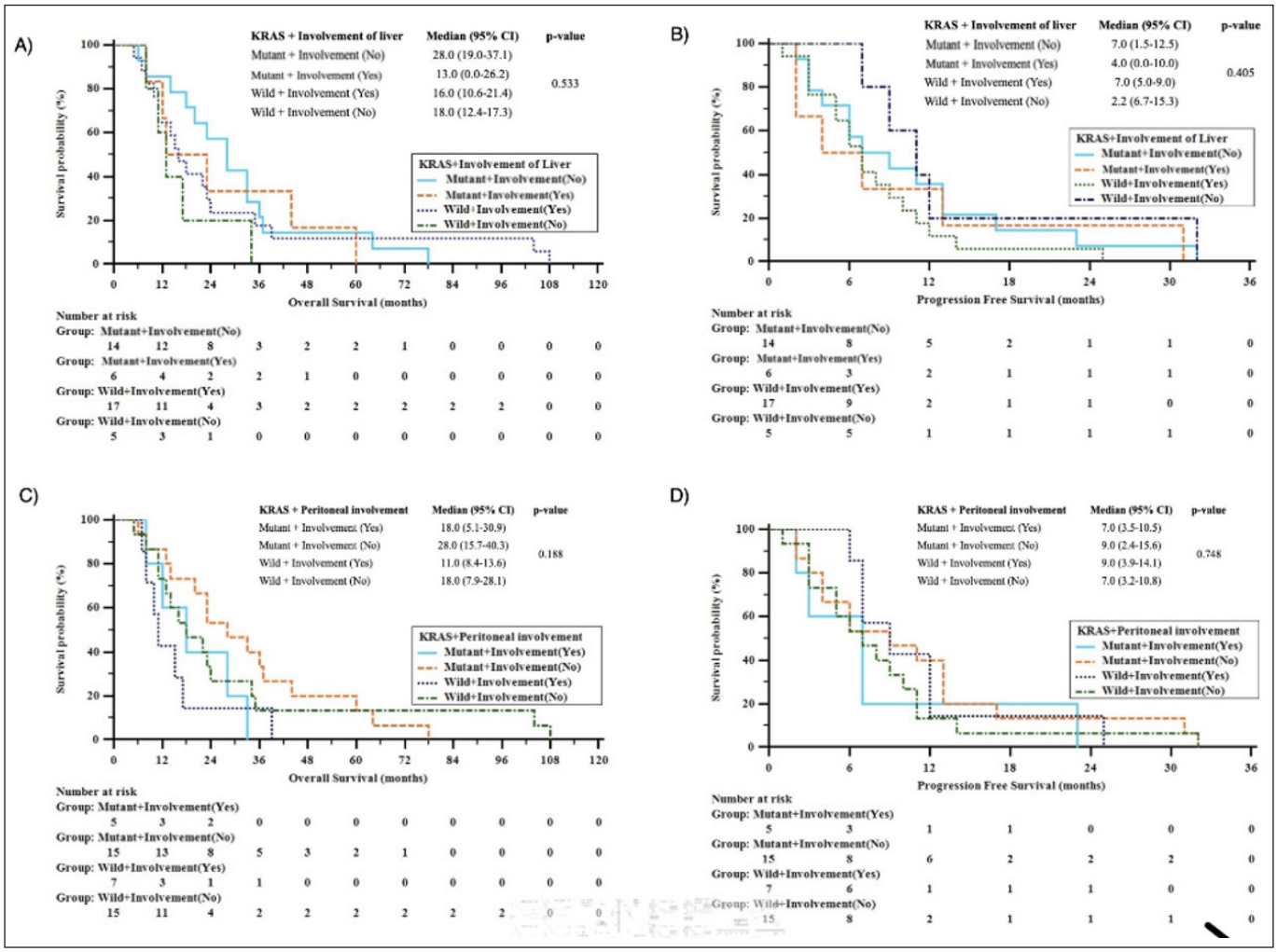

Figure 3. KRAS+ liver involvement and peritoneal involvement

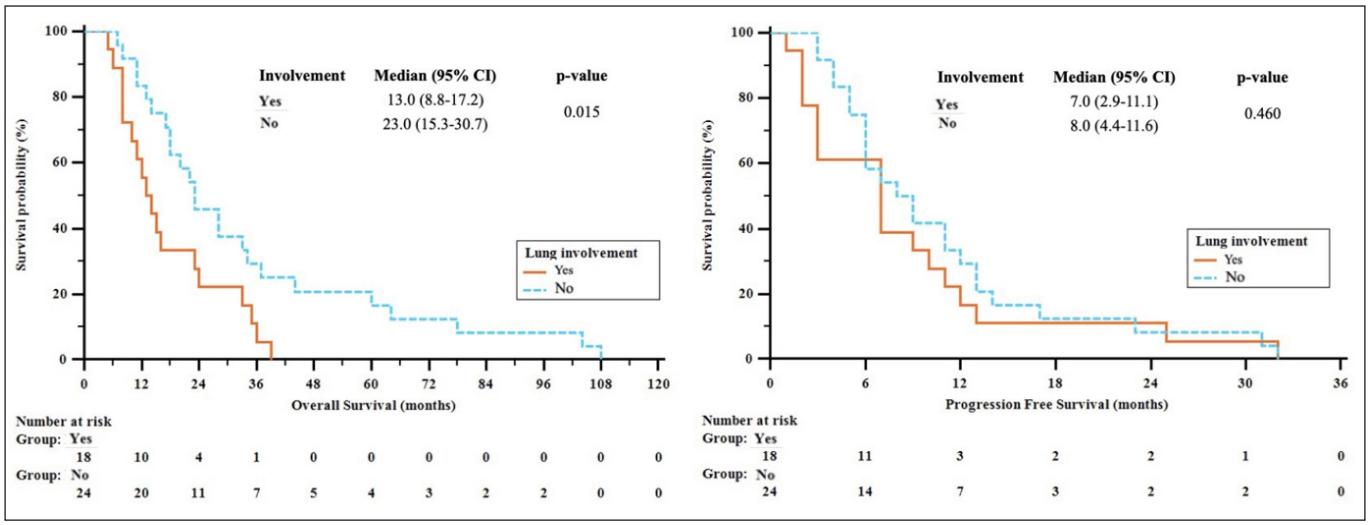

Figure 4. KRAS+ lung involvement

DISCUSSION

Despite the advantages provided by the development of imaging technology in diagnosis and follow-up processes and the success rates achieved in treatment, the incidence of colorectal cancer, like all other cancers, is gradually increasing all over the world with the increasing population.[2] This situation has led to an increased interest in the epidemiology of colorectal cancers and the factors determining the treatment process and prognostic and predictive factors in recent years. Since concomitant metastases determine morbidity and mortality as well as primary cancer, studies determining cancer treatment processes aim to understand possible metastasis mechanisms. When we look at colorectal cancer and its

concomitant metastases, we see that approximately 20% of patients have concomitant metastases at the time of diagnosis, since no obvious symptoms are observed in the early stages of colorectal cancer.[3] Half of patients die, usually in the first years after diagnosis, mostly due to the consequences of metastatic disease.[6] Large population studies have shown that approximately one-third of patients diagnosed with colorectal cancer develop liver metastases during follow-up. In our study, 73.8% of patients had liver metastases, 57.1% had lung metastases, 28.6% had peritoneal metastases, and 28.7% had other metastases (**Table**). There is a strong correlation between survival in colorectal cancer and the site of metastasis. Deaths due to colorectal cancer are usually due to metastatic spread to distant sites. In general, peritoneal metastases are less frequently diagnosed. Therefore, the true incidence of peritoneal metastases is uncertain but has been reported to be as high as 40-80% in autopsy series. Patients with peritoneal metastases have the worst prognosis of all patient groups.[7] Many studies have found that patients with peritoneal metastases have poorer survival rates than those with metastases to other sites, with untreated patients dying within months.[8-11] Studies showing that the median survival rate of colorectal cancer patients with peritoneal metastases decreased by 30% were supported by subsequent studies and reported median survival times between 10.4 and 23.9 months. Another prospective study reported a median survival of 21.5 months and a median time to progression of 4.4 months.[12,13] Our own study also supports previous studies, and we found the median overall survival to be 12 months in patients with peritoneal involvement and 23 months in patients without peritoneal involvement, and there was a statistically significant difference between the two groups (p: 0.041<0.05). According to our analysis, patients with peritoneal involvement survived less than those without. In our disease-free survival analysis, the median progression-free survival of patients with and without peritoneal involvement was equal at 7 months, and no statistically significant difference was observed (p<0.05) **Figure 1**.

The liver is the most common site of colorectal cancer metastasis, followed by other metastatic sites. Since most mesenteric venous drainage drains into the hepatic portal vein system, more than 50% of patients present with liver metastases.[14] In the relationship between colorectal cancer and liver metastases, studies have shown that approximately 15-25% of patients have distant metastases at the time of initial diagnosis, and the remaining 18-25% of patients develop distant metastases within 5 years of initial diagnosis, resulting in approximately 50%-70% of patients with colorectal cancer developing metastases in the liver. In colorectal cancer patients with liver metastases, median survival without treatment is 5 to 20 months, and the 5-year survival rate is 11%, which is the best prognosis for patients presenting with local metastases.[15,16] However, over the years, improved resection and treatment protocols have resulted in favorable outcomes in liver metastatic cases, resulting in median survival times of over 30 months (19-50 months) and progression-free survival times ranging from 6-12 months.[17] In our patient population, we found that the median overall survival was 22 months in patients with liver involvement and 15 months in patients without liver involvement, and there was no statistically significant difference between them (p=0.399>0.05). The median progression-free survival was 9 months in patients with liver involvement and 7 months in patients without liver involvement, and there was no statistically significant difference between them (p=0.396>0.05). The overall median survival time and progression-free survival times in our study are consistent with other studies. The overall and disease-free survival curves according to the presence of liver and peritoneal involvement in our study participants are shown in **Figure 1**.

Tabel. Distribution of demographic and clinical characteristics of participants

Characteristics (N=42)	n (%) or Mean (Min-Max)
Gender	
Male	26 (61.9)
Female	16 (38.1)
Age year	59 (21-81)
Localization	
Colon	21 (50)
Rectum	19 (45.2)
Kolorectal	2 (4.8)
Subtype	
Adenocarsinoma	38 (90.5)
Mucinous	4 (9.5)
KRAS gen	
Mutant	20 (47.6)
Wild	22 (52.4)
Liver involvement	31 (73.8)
Peritoneal involvement	12 (28.6)
Lung involvement	24 (57.1)
Other involvement	
Overall Survival, month	19 (5-108)
Progression- free Survival, Month	7 (1-32)

To date, various hypotheses have been put forward regarding the development of colorectal cancer. As in other cancer types, many genetic and environmental factors are responsible for the development of colorectal cancers. Kirsten rat sarcoma viral oncogene (KRAS) is a small proto-oncogene that binds to a protein involved in the regulation of cellular responses to many extracellular stimuli.[18,19] It is now widely accepted that sporadic colorectal cancers usually arise from preneoplastic lesions through inactivation of tumor suppressor genes

and activation of oncogenes.[20] Colorectal cancers are predominantly KRAS mutant types. In mutant types, the tumor is more aggressive, and survival is less likely. In addition, the presence of mutations develops resistance to treatment. Therefore, the determination of KRAS mutation status is valuable in the treatment of patients with this condition.[21,22]

Compared with KRAS wild-type colorectal cancer, KRAS mutant colorectal cancer has a different biological behavior and therapeutic approach.[21] Although the proven predictive value of KRAS mutations has long been accepted, the prognostic value of these mutations is still under evaluation. Since colorectal cancer continues to be an important public health problem, it is of great importance to determine the parameters affecting its prognosis. To mention some important studies, the RASCAL study has shown that the presence of mutations increases the likelihood of recurrence and death.[23] Many studies have shown that stage III patients with KRAS mutations exhibit significantly worse disease-free survival than those with wild-type KRAS, which may be partly explained by the effect of KRAS mutations on prognosis.[24-28] Another study found that patients with any gene mutation may develop resistance to anti-EGFR therapy and have worse outcomes and different metastatic patterns compared to those with wild-type genes.[29] Foltran and colleagues showed that patients with any oncogene mutation had worse survival rates compared to those with wild-type.[30] However, there are also studies with different results. Dinu and colleagues found that the status of the KRAS gene had no prognostic significance.The overall and disease-free survival curves according to the KRAS gene type included in our study are shown in **Figure 2**. In our study, the median overall survival of mutant genes was 15 months, while the median survival of wild-type genes was 23 months, which is consistent with a recent study.[31] However, we did not find a statistically significant difference between the two genes in terms of overall survival in our study (p=0.438>0.05) **Figure 2**. In terms of disease-free survival, the median progression times of both mutant and wild-type were equal at 7 months, and no statistically significant difference was observed between them (p<0.05). There are studies that have found that patients with KRAS mutations are more likely to have peritoneal metastases, liver-peritoneal metastases, and multiple organ metastases compared to all wild-type Kang and colleagues found that in tumors with any gene mutation, the KRAS mutation metastasized more frequently to the peritoneum and liver-peritoneum compared to all wild-type.[3] Furthermore, KRAS or BRAF mutations associated with a poor prognosis compared to their wild-type counterparts were also reported by Liu and colleagues.[32] Ucar and colleagues have shown that multiple KRAS mutations are associated with a better prognosis compared to single mutations.[33] There are many studies on the median survival and progression-free survival times in the presence of KRAS mutations.[34-36] However, to the best of our knowledge, our study is the first to investigate the median survival and progression-free periods in the presence of liver, peritoneal, and lung metastases in patients with KRAS mismatch. The overall and disease-free survival curves of the participants in our study according to the presence of liver and peritoneal involvement according to KRAS gene type are shown in **Figure 3**. In terms of overall survival, the median survival was 13 months for those with mutant type and liver involvement and 28 months for those without, while it was 16 months for those with wild type and liver involvement and 18 months for those without. However, no statistically significant difference was observed between them (p=0.533>0.05). In terms of disease-free survival, the median progression time was 4 months for mutant type and those with liver involvement and 7 months for those without, 2 months for wild type and those with liver involvement and 7 months for those without, and no statistically significant difference was observed between them (p: 0.405>0.05). Similarly, when the presence of peritoneal involvement was analyzed according to KRAS gene types, median survival was found to be 18 months in patients with mutant type and peritoneal involvement and 28 months in patients without peritoneal involvement. While the median survival was 11 months in patients with wild-type and peritoneal involvement, it was 18 months in patients without peritoneal involvement. However, no statistically significant difference was observed between the groups (p<0.05). In disease-free survival, median progression-free survival was 7 months in patients with mutant gene and peritoneal involvement and 9 months in those without, while median progression-free survival was 9 months in patients with wild-type gene and peritoneal involvement and 7 months in those without, and no statistically significant difference was observed (p:0.748>0.05). If we look at the frequency of lung involvement according to the KRAS gene type of the participants, the overall and progression-free survival curves are shown in **Figure 4**. In terms of overall survival, the median survival time for patients with mutant type and lung involvement was 12 months and 28 months for those without, while the median survival time for patients with wild type and lung involvement was 13 months and 18 months for those without. There was no statistically significant difference between the groups (p=0.100>0.05). In disease-free survival, the median progression time was 7 months for patients with wild-type lung involvement and 8 months for patients without lung involvement. The median time

to progression was 7 months for mutants and those with lung involvement and could not be calculated for those without lung involvement because the progression rate did not reach 50%. No statistically significant difference was observed (p=0.804>0.05).

Study Limitations

The patients selected in this study were conducted with a limited number of patients in a single center, and since it is a retrospective study based on the data in their files, the study was limited to these two factors.

CONCLUSION

Our study is consistent with other studies showing that the KRAS mutation has a negative effect on overall survival results. However, in the retrospective analysis we conducted on the relationship with metastatic foci to investigate the value of KRAS genes as a prognostic factor, median survival and progression-free survival times were shortened in the liver metastatic group, the group with periteneal involvement, and the group with lung involvement in KRAS mutant patients, but the results were not statistically significant. Therefore, although the predictive and treatment-guiding contribution of KRAS genes has been proven many times, its prominent role as a prognostic factor could not be proven in our study as in some other studies. In this sense, our study aims to contribute to studies questioning the real role of KRAS genes as prognostic markers. Multicenter studies are needed to diversify the predictive and prognostic factors of colorectal cancers and to clarify the contribution of KRAS genes in this regard.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Clinical Researches Ethics Committee (Date: 21.12.2022, Decision No: 2022-12/5).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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