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Araştırma Makalesi / Research Article

Comparison of KRAS Mutation Status with Clinical Parameters in Colon Adenocarcinoma

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Abstract

KRAS mutations are mutually exclusive with other activating mutations on EGFR pathway. Detection of KRAS mutations associated with tumorigenesis, predicates the lack of other mutations on the same pathway and shows that the application of targeted therapy approaches which target other proteins in EGFR-MAPK pathway ineffective. In this study, frequency of KRAS mutations in colorectal cancer and relationship between KRAS mutation status and other clinical features were assessed. KRAS mutations were detected in 47,7% of the cases included in our study. We determined that 76% of the mutations were located in codon 12, 9% of the mutations were located in codon 13, 9% of the mutations were located in codon 61 and 6% of the mutations located in codon 117 or codon 146. Determination of mutation rates and association of mutations with clinical features for different populations are important for planning of the treatment strategies nationwide. In our study, we have demonstrated that KRAS mutation status and clinical features associated with KRAS mutation is in accordance with the literature. We have determined that there is statistically significant correlation between grade and KRAS mutation status.

Keywords: Colorectal cancer, KRAS oncogene, Anti-EGFR treatment

Kolon Adenokarsinomlarında KRAS Mutasyon Durumunun Klinik Veriler ile Karşılaştırılması

Öz

KRAS mutasyonu EGFR yolağındaki diğer aktifleştirici mutasyonlarla birbirini dışlayan özellik göstermektedir. Tümörijenez ile bağlantılı KRAS mutasyonlarının tespiti, aynı yolak üzerinde başka mutasyonların yokluğunu da büyük ölçüde göstermekte ve dolayısıyla EGFR-MAPK yolağı üzerindeki başka proteinleri hedefleyen akıllı ilaçların kullanımının fayda sağlamayacağını göstermektedir. Bu çalışmada kolorektal kanser vakalarında KRAS mutasyonu görülme sıklığı ve mutasyon durumunun diğer klinik veriler ile ilişkisi incelenmiştir. KRAS mutasyonu çalışmamıza dahil edilen vakaların %47,7'sinde tespit edilmiştir. KRAS mutasyonlarının %76'sının 12. kodonda, %9'unun 13. kodonda, %9'unun 61. kodonda ve %6'sının 117. veya 146. kodonda gerçekleştiği tespit edilmiştir. Farklı populasyonlarda değişebilen mutasyon oranlarının ve mutasyonların klinik özelliklerle ilişkisinin tespit edilmesi ulusal tedavi stratejilerinin planlanması açısından büyük öneme sahiptir. Çalışmamızda KRAS mutasyon durumunun ve KRAS mutasyonu ile bağlantılı klinik özelliklerin literatür ile uyumlu olduğu gösterilmiştir. Çalışmamızda KRAS mutasyon durumu ile tümör grade'i arasında istatistiki olarak anlamlı korelasyonun varlığı tespit edilmiştir.

Anahtar Kelimeler: Kolorektal kanser, KRAS onkogeni, Anti-EGFR tedavisi

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1. Introduction

Colorectal cancer was the third most common cancer in the World and led second highest cancer related mortality in the year of 2018 (Wong et al., 2021). Extension of the lifespan in World, increases in the average body mass index and tobacco and alcohol consumption, obesity and negative changes in diet led the increase of incidence of colorectal cancer (Lee et al., 2020; Rawla et al., 2019).

Epidermal Growth Factor Receptor (EGFR) and pathways that EGFR is involved played significant roles in development of Colorectal Carcinoma (London and Gallo, 2020). For that reason, EGFR become an important target molecule in cancer treatment (London and Gallo, 2020**;** Belli et al., 2020). EGFR is a transmembrane protein that spans both sides of the plasma membrane. When specific ligands bind to EGFR, EGFR transmit signals coming outside of the cell to cytoplasm. When EGFR is activated, KRAS and PI3K pathways are activated subsequently directly or indirectly via adaptor proteins harboring SH2 domains (Vitiello et al., 2019). When these pathways are activated, they activate other proteins and transmit extracellular signals to the nucleus where gene expression occurs and these signals were to be effective on cell proliferation and cell survival.

KRAS protein is a protein localized at the inner surface of the plasma membrane with GTPase activity and activated by EGFR. While KRAS protein is activated when bound to GTP and KRAS become inactivated when GTP is hydrolyzed to GDP (Menyhárd et al, 2020). KRAS mutations are one of the most frequent mutations observed in human cancers and 30% to 40% of colorectal carcinoma cases harbors KRAS mutations (Prior et al., 2020, Timar and Kashofer, 2020). More than 90% of KRAS mutations associated with tumorigenesis are placed in 12th and 13th codons which corresponds to GTPase domain of the KRAS gene (Peeters et al., 2013). Apart from mutation in 12th and 13th codon mutations, there are also mutations that confer cancer phenotype located in codon 61, codon 117 and codon 146 (Imamura et al., 2014).

With the discovery of the role of EGFR pathway in tumorigenesis, monoclonal antibodies which target EGFR is also started to be developed (Hobbs et al., 2016; Cai et al., 2020). Monoclonal antibodies like Cetuximab and Panitumumab which competitively bound to EGFR, prevent the functioning of all MAPK pathway by blocking the binding of natural ligands of EGFR and also preventing internalization of EGFR from the plasma membrane (Li et al., 2020; García-Foncillas et al., 2019).

If a mutation occurs in KRAS which located downstream of the EGFR, blocking EGFR with a monoclonal antibody do not lead inhibition of cancer phenotype and RAS pathway remains activated independent of EGFR. Since KRAS gene is frequently mutated in all types of cancer including colorectal cancer, routine KRAS mutation testing for determination of the efficiency of anti-EGFR agents is performed in metastatic colorectal cancer. KRAS and EGFR were mutually exclusive

mutations and if RAS mutation (KRAS, NRAS) is detected in a patient, lack of EGFR mutation can be deducted (Sanchez-Ibarra et al., 2020).

Published data related to KRAS mutation status on Turkish cohorts are rare and analysis of KRAS mutation status with other clinical features were very valuable for understanding the nature of the disease and also planning of local treatment strategies since it is known that mutation frequencies change among cohorts of different populations. We aim to contribute the accumulation of knowledge related to tumorigenesis and cancer treatment by comparing KRAS mutations status and type with other clinicopathological parameters with this study.

2. Materials and Methods

In this study, pathology reports which belong to 44 Colonic-type adenocarcinoma cases which were admitted to Haydarpaşa Numune Training and Research Hospital between the dates of 21.07.2020 to 15.12.2020 on which KRAS mutation analysis were performed, were retrospectively analyzed.

Isolation of genomic DNA from FFPE specimen blocks was performed using the AmoyDx FFPE DNA Kit with nucleic acid purification spin columns (Amoy Diagnostics, Xiamen, China). By spectroscopy analysis, all purified DNA and RNA samples were judged to be of high quality for mutation analysis. The concentration of the DNA isolated from specimens was measured by a Merinton SMA4000 spectrophotometer (Merinton Inc., Beijing).

Pathologically significant mutations in KRAS gene were analyzed by AmoyDx KRAS Mutation Detection Kit which scans 19 mutations at 12th, 13th, 59th, 61st, 117th and 146th codons at the KRAS gene which based on ARMS-PCR and Reverse Transcriptase Polymerase Chain Reaction. Mutation specific primers labeled with FAM and HEX dyes were used in polymerase chain reaction.

35 microliter mastermix and 0,3 microliter Taq Polymerase enzyme was added to every reaction mix and then 5 microliter of DNA of the concentration of 2 nanogram/microliter is added to every mix, thus a Real Time Polymerase Chain Reaction was conducted with a total of 10 nanogram DNA.

Polymerase Chain Reaction was conducted according to the conditions stated in **Table 1.**

Ct value smaller than 26 is accepted as strong positive and means %5 or more mutant DNA in total DNA and CT value greater than 26 is accepted as weak positive and means %5 or lower mutant DNA.

Data which was used for statistical analysis was generated from RAS mutation scanning reports together with age, gender, tumor localization and other patient related data.

Table 1: Conditions of Polymerase Chain Reaction

Stage	Cycle No	Temperature	Time	Data Collection
1	1	95◦C	5 min	
2	15	95◦C	25 sec	
		64◦C	20 sec	
		72◦C	20 sec	
3	31	93◦C	25 sec	
		60◦C	35 sec	FAM and HEX
		72◦C	20 sec	

Since all the variables in this data set were categorical variables, Chi-square independence test is performed upon this data set. The table used for these variables was a table which includes every two variables and frequencies.

The hypothesis was set as follows:

H0: Variables on Rows and Column were independent. (There is no relationship between Row and Column Variables.)

H1: Variables on Rows and Column were dependent. (There is relationship/s between Row and Column Variables.)

The method to be applied varies according to theoretical frequencies calculated for every cell in cross table. Generally, according to least theoretical frequency for 2x2 tables;

• if the least theoretical frequency > 25, Pearson Chi-square test

• if the least theoretical frequency is between 5 and 25 Yates' Chi-square test

• if the least theoretical frequency is <5 Fisher Exact Test

were used.

If the percentage of theoretical frequencies calculated for each cell which were lower than 5 is smaller than 20% Pearson chi-square test is used, if the percentage of theoretical frequencies calculated for each cell which were lower than 5 is larger than 20% Exact test was used.

In this data set Pearson Chi-square test was used for mutation status versus gender analysis and Fisher Exact Test was used for other variables.

All the statistical analysis is conducted by IBM SPSS Statistics for Windows, version 28.0.0.0 (190) (IBM Corp., Armonk, N.Y., USA).

3. Findings and Discussion

Our study group is comprised of 44 cases which were diagnosed as "adenocarcinoma colonic type" between the dates of 21.07.2021 and 15.12.2020.

56,8% (n=25) of the cases were male and 43,2% (n=19) of the cases were female. 47,7% (n=21) of the cases were KRAS positive and 52,3% (n=23) of the cases were KRAS negative (**Table 2**).

When cases were stratified according to age groups of age of 50 or lower, age between 51 and 70, and age greater than 70, it was observed that 18,2% (n=8) of the case were below the age of 50, 59,1% of the cases were between 51 and 70 and 22,7% of the cases were age of greater than 70 (**Table 2**).

Table 2: Patient characteristics and the association between KRAS mutational status and clinicopathologic parameters

	All	KRAS Wild Type	KRAS Mutant	P value
	N	%	%	
Gender				0,361
Male	25	60%	40%	
Female	19	42%	58%	
Age				0,540
<51	8	37,5%	62,5%	
51-70	26	57,7%	42,3%	
>70	10	50%	50%	
Tumor Location				0,4368
Colon	24	50%	50%	
Rectum	10	40%	60%	
Other	10	70%	30%	
Grade				0,0857
Well Differentiated	7	85,8%	14,2%	
Moderately Differentiated	23	43,5%	56,5%	

Average age of the cases included to this study is 61,4, while the average age of male patients were 62,6 and average age of female patients were 59,9.

KRAS positivity among male patients was 40% (n=10) and among female patients was 57% (n=11) (p=0.3610000).

When KRAS mutation status is assessed according to age groups, KRAS positivity ratio was 62,5% in the age group lower than 51, KRAS positivity ratio was 42% age group 51-70 and 50% in the age group greater than 70 (p= 0.5407361).

When patients enrolled to this study is stratified according to age groups of < 51, 51-70,> 70, age group stratification for males were found to be %16 (n=4), %64 (n=16) and %20 (n=5) and for females were found to be %21 (n=4), %52 (n=10) and %26 (n=5) (p= 0.7613762) respectively.

When distribution of mutations was assessed and when both mutations of the two cases which harbor two mutations rather than one were taken into consideration, it was determined that 26% of the mutations were G12D, 22% of the mutations were G12A, 22% of the mutations were G12V, 8,6% of the mutations were Q61X, 8,6 % of the mutations were K117X or A146X, 4,3% of the mutations were G12S, 4,3% of the mutations were G13C and 4,3% of the mutations were G13D. Two different

mutations were detected in two cases and tumors harboring two KRAS mutations reflects the tumor heterogeneity (**Table 3**).

Table 3: Distribution of KRAS Mutation Types

KRAS Mutation Type	Percentage %
G12D	26%
G12A	22%
G12V	22%
Q61X	8,6%
K117X or A146X	8,6%
G12S	4,3%
G13C	4,3%
G13D	4,3%

When mutations were separated according to codons which they affected, it was determined that 74% of the mutations were located in codon 12, 8,6% of the mutations were located in codon 13, 8,6% of the mutations were located in codon 61, 8,6% of the mutations were located in codon 117 or codon 146.

When distribution of mutation types with respect to age groups was analyzed, it was observed that 100% of mutation located in codon 117 or codon 146 and 50% of mutations located in codon 61 is placed in the group of patients whose ages were below 51. Moreover, two tumors which harbor two distinct mutations were located in the group of patients whose ages were below 51. In the group of patients whose ages varies between 51 and 70, all mutations effects codon 12 except one codon 13 mutation. In the group of patients whose ages were greater than 70, all mutations except one mutation effects codon 12.

Among the cases which were included of this study, 54,5% of the cases were obtained from Colon, 22,7% of the cases were obtained from Rectum, 9% of the cases were obtained from Liver, 2,27% of the cases were obtained from Brain, 2,27% of the cases were obtained from Omentum, 2,27% of the cases were obtained from Abdomen, 2,27% of the cases were obtained from Lymph Node and 2,27% of the cases were obtained from Bone.

When localization of the cases included in this study was compared with KRAS mutation status, it was observed that 57% of the KRAS positive cases were originated from Colon, 28,5% of the KRAS positive cases were originated from Rectum, 14,3% of the KRAS positive cases were originated from Other Organs. For the case of KRAS negative cases, it was observed that 52,2% of the KRAS positive cases were originated from Colon, 17,4% of the KRAS positive cases were originated from Rectum, 30,4% of the KRAS positive cases were originated from Other Organs

When cases included in this study were separated according to KRAS mutation status and tumor grade was compared, it was observed that 93% of the cases were moderately differentiated and 7% of the cases were well differentiated for KRAS positive cases, while it was observed that 62,5% of

the cases were intermediately differentiated and 37,5% of the cases were well differentiated for KRAS negative cases (p=0.0859770).

When grade of the tumor specimens and age of the cases were compared, it was observed that average age of moderately differentiated tumors were 63,9 (median 64) and average age of well differentiated tumors were 59,28 (median 64) (p=0.2305158).

Statistical analysis results of variables in this study are provided in **Table 4**. Moreover, we have determined that there is statistically significant correlation between grade and KRAS mutation status (p=0.0859770) and age group versus KRAS mutation type (p= 0.0814710) when type one error is taken as 0,1.

Table 4: Results of Statistical Analysis

Variables	p.value	Test
Sex-Age	0.7613762	Fisher-Freeman-Halton Exact
Sex-Localization	0.3596705	Fisher-Freeman-Halton Exact
Sex-Grade	0.6378073	Fisher's Exact
Sex-KRAS mutation type	0.1425540	Fisher-Freeman-Halton Exact
Sex-Mutation Status	0.3610000	Pearson Chi-Square
Sex-Codon	0.8540469	Fisher-Freeman-Halton Exact
Sex-Side	0.0877193	Fisher's Exact
Age-Localization	0.4156319	Fisher-Freeman-Halton Exact
Age-Grade	0.2305158	Fisher-Freeman-Halton Exact
Age-KRAS mutation type	0.0814710	Fisher-Freeman-Halton Exact
Age-KRAS Mutation Status	0.5407361	Fisher-Freeman-Halton Exact
Age-Codon	0.1403181	Fisher-Freeman-Halton Exact
Localization-Grade	0.2281776	Fisher-Freeman-Halton Exact
Localization-KRAS mutation type	0.6075764	Fisher-Freeman-Halton Exact
Localization-KRAS Mutation Status	0.4367983	Fisher-Freeman-Halton Exact
Localization-Codon	0.9351319	Fisher-Freeman-Halton Exact
KRAS mutation type -Grade	0.9195402	Fisher-Freeman-Halton Exact
Grade-KRAS Mutation Status	0.0859770	Fisher's Exact
Grade-Codon	1.0000000	Fisher-Freeman-Halton Exact

Colorectal cancer is developed with continuous proliferation and survival (evasion of apoptosis) of cells as a result of accumulation of various activating mutations, loss of function of tumor suppressor proteins and activation of proto-oncogenic proteins like KRAS (László et al., 2021). Surgical intervention cannot be applied to metastatic colorectal cancer. When surgical intervention is not enough, anti-EGFR treatment where EGFR monoclonal antibodies such as cetuximab or panitumumab are used (Li et al., 2020). When targeted therapy approaches are employed, anti-EGFR agents bind competitively to EGFR, thus prevents continuous activation of MAPK pathway by EGFR (Russo et al., 2015). But, if the activation of the MAPK pathway is caused by mutations on KRAS which is downstream of EGFR, application of agents that specifically target EGFR is not beneficial for the patient (Lee et al., 2018). Thereby, routine KRAS mutation testing is applied to colorectal cancer patients before determination of the treatment which will be applied to the patients.

KRAS mutation status and its relationship between various clinical features have been investigated in Turkey. In a recent study of Babat and colleagues, KRAS mutation status of colorectal cancer patients were investigated and 47% of the patients which KRAS mutation data available is found to harbor KRAS mutations (Babat et al., 2021). In another recent study, Uçar and colleagues, KRAS mutation ratio is found to be 45% and 4,5% of the KRAS positive cases harbors double mutations (Uçar et al., 2020). In a study published by Varlı and colleagues in 2020, KRAS mutation ratio is observed as 37,5% of patients and 88,9% of the mutations were found to be located in Codon 12 and 11,1% of mutation were found to be in Codon 13 (Varlı et al., 2020). In study conducted by Eraslan and colleagues, KRAS mutation ratio was observed as 37,5% (Eraslan et al., 2021). While there are some studies including KRAS mutation status were published, in our study we aimed to determine the KRAS mutation frequency in colorectal cancer in single institute in Turkey and investigate the relationship between KRAS mutation status with a unique set of clinical features

KRAS mutation status and its relationship with clinical parameters is assessed in several nationwide studies all around the World. In a recent NGS based study of Belardinilli and colleagues from Italy, KRAS mutation ratio is found 39,5 % of the patients and it is demonstrated the mutual exclusivity of KRAS, NRAS and BRAF mutations (Belardinilli et al., 2020). In a recent Chinese study, the clinicopathologic features and KRAS mutation status was compared. In the study of Chang and colleagues, KRAS positivity ratio was determined as 47,56% and KRAS mutation ratio in well-differentiated patients was found to be significantly higher than moderately differentiated patients (Chang et al., 2021). In a study conducted by Ikoma and colleagues from Japan, KRAS mutation ratio is determined as 48% (26% Codon 12, 17% Codon 13 and 5% non-Exon2 mutations) and while no correlation between KRAS mutation status with age and gender were found (Ikoma et al., 2021). In a study conducted by Abudabous and colleagues from Libya on 34 colorectal cases, KRAS mutation ratio is found to be 38,2%, while frequent mutations were G12D (46%), G12V (30,8%), G12C (15,4%) and G13D (7,7%) respectively and it was determined that well differentiated tumors more likely to harbor KRAS mutations (Abudabous et al., 2021).

Our results are similar with the results in the literature. In our study KRAS mutation ratio is determined as 47% which is in accordance with results listed in literature (**Table 5**)

Table 5: KRAS Mutation Ratio in Literature

KRAS Mutation Ratio in Literature	
Study	**Percentage %**
Balardinilli et al., 2020	39,4%
Chang et al., 2021	47,6%
Ikoma et al., 2021	48%
Babat et al., 2021	47%
Uçar et al., 2020	45%
Eraslan et al., 2021	37,5%
Abudabous et al., 2021	38,2%
Our Study	47%

When the relationship between gender and KRAS mutation status is assessed in our study, we have determined that KRAS mutation ratio of %40 (n=10) in male and %57 (n=11) in female patients. The percentage difference between male and female patients is not statistically significant and this difference maybe aroused from limited sample size of our study. When published results were assessed, in a study conducted by Chang and colleagues in 2021 on colorectal cancer patients, it was determined that %45,7 of female patients and %48,8 of male patients carried KRAS mutation (Chang et al., 2021). In a study conducted by Kwak and colleagues in 2018 on colorectal cancer patients, it was determined that %46 of female patients and %34 of male patients carried KRAS mutation (Kwak et al., 2018) (**Table 6**).

Table 6: KRAS Mutation Ratio according to Gender

	Chang et al., 2021	Baskin et al., 2014	Kwak et al.,2018	Our Study
Male	48,8%	31,3%	34%	40%
Female	45,7%	33,3%	46%	57%

When age of the patients with colorectal cancer is assessed, it was determined that Colorectal cancer is most frequently observed between ages of 50 and 70 and age threshold for "young-colorectal cancer" is still discussed (Davis et al., 2011). In a study conducted by Jianfei Fu and colleagues in 2014, threshold age for "young-colorectal cancer" is determined as 35 and below and the ratio of colorectal cancer patients under the age of 35 is determined as 5,7% (Fu et al., 2014). In our study percentage of patients under 35 is determined as 4,55%.

In our study, when KRAS mutation status according to age groups was assessed, KRAS mutation status is highest in the group of patients whose age is below 51 with 62,5%. KRAS mutation ratio is lowest in a group of patients whose age varies between 51 and 70 and 50% in patients whose age is higher than 70.

When the distribution of gender across age groups was assessed in our study, in the age groups of 50 or below, 51-70 and 71 and above, for males 15,3% (n=4), 64% (n=16) and 20% (n=5) and for females 21% (n=4), 52% (n=10) and 26% (n=5) respectively.

In a study conducted by Hsu and colleagues in 2016, tumor localization of colorectal cancer patients was evaluated and tumors located in colon and tumors located in rectum was compared, colon localized tumor versus rectum localized tumor ratio was found to be 60,4% versus 39,6% (Hsu et al., 2016). 54,5% of the samples included to our study is obtained from colon, %22 of the samples included to our study is obtained from rectum. When only colon originated and rectum originated tumors were compared, it was observed that 70,6% of the tumors were colon originated and 29,4% of the tumors were rectum originated. In our study, like the study of Hsu and colleagues, colon originated tumors were more numerous than rectum originated tumors.

Most frequent metastasis location of colorectal cancer is liver. In a study conducted by Holch and colleagues in 2017, 71% of the metastatic samples of colon tumors and 60% of the metastatic samples of rectal tumors were found in liver (Holch et al., 2017). In a sudy conducted by Riihimäki and colleagues, 62% of colon adenocarcinoma cases were found to metastasize in liver (Riihimäki et al., 2016). In our study when origin of tumor specimen is analyzed and when tumors originated from the colon and rectum were excluded, we found that 39,8% of the non-colon and rectum tumors were originated from liver. The difference between the reported results may arise from the limited size of our study group.

In a study conducted by Awidi and colleagues in 2019, KRAS mutation frequencies were detected as G12D (19,6%), G12A (17,4%), G12T (14,1%), G12V (10,7%), G13D (7,6%), G13A (6,5%), G12S (3,3%), G12C (2,2%), K117N or A146V (10,85%) and Q61X (5,4%) (Awidi et al., 2019). In a study conducted by Baskin and colleagues in 2014, ratio of KRAS mutations in codon 12 and codon 13 were evaluated (Baskin et al., 2014). In this study 93,4% of mutations are found to be in codon 12 and 2% of the mutations were found to be codon 13. Mutation frequencies were detected as G12D (12.2%), G12V (10.2%), G12C (4.1%), G12R (2%), and G13D (2%) respectively in this study. In our study when mutation distribution was assessed and cases which harbor two mutations were included, mutation frequencies were detected as 26% for G12D, 22% for G12A, 22% for G12V, 8,6% for Q61X, 8,6% for K117X or A146X, 4,3% for G12S, 4,3% for G13C and 4,3% for G13D. Two different mutations were detected in two cases and one tumor harboring two mutations represent tumor heterogeneity. Mutation frequency obtained from our study is in accordance with current literature (**Table 7**).

In a study conducted by Imamura and colleagues in 2014 where KRAS mutations in codons associated with resistance against anti-EGFR treatment, it was observed that 68% of the mutations were placed in codon 12, 22,7% of the mutations were placed in codon 13, 3,8% of the mutations were placed in codon 61 and 7,9 of mutations were located in codon 146 (**12**). In our study, it was observed that 74% of the mutations were placed in codon 12, 8,6% of the mutations were placed in

codon 13, 8,6% of the mutations were placed in codon 61 and 8,6% of the mutations were placed in codon 117 or codon 146. Our results are in accordance with current literature.

Table 7: Distribution of KRAS Mutations in Literature

	Mutations	Baskin et al., 2014	Awidi et al., 2019	Our Study
Codon 12	G12D	39%	19,6%	26%
	G12C	13,4%	2,1%	
	G12V	33%	10,1%	22%
	G12R	6,5%		
	G12A		17,4%	22%
	G12S		3,3%	4,3%
	G12T		14,1%	
	G12X			
Codon 13	G13D	6,5%	7,6%	4,3%
	G13A		6,5%	
	G13R			
	G13C			4,3%
	G13X			
Codon 61	Q61X		5,4%	8,6%
Codon 117 or Codon 146	K117X or A146X		10,9%	8,6%

When distribution of mutations types with respect to age groups was analyzed, it was observed that 100% of mutation located in codon 117 or codon 146 and 50% of mutations located in codon 61 is placed in the group of patients whose ages were below 51. Moreover, two tumors which harbor two distinct mutations were located in the group of patients whose ages were below 51. In the group of patients whose ages vary between 51 and 70, all mutations effects codon 12 except one codon 13 mutation. In the group of patients whose ages are higher than 70, all mutations except one mutation effects codon 12. Whether the stratification of mutations was random shall be further investigated.

When cases included in this study were separated according to KRAS mutation status and tumor grade was compared, it was observed that 93% of the cases were moderately differentiated and 7% of the cases were well differentiated for KRAS positive cases, while it was observed that 62,5% of the cases were intermediately differentiated and 37,5% of the cases were well differentiated for KRAS negative cases. We have demonstrated that there is statistically significant correlation between grade and KRAS mutation status where well differentiated tumors are associated with lower KRAS mutation rate when Type 1 error value is taken as 0,1.

4. Conclusions and Recommendations

Published data related to KRAS mutation status and its relationship between clinical features on Turkish cohorts are rare and analysis of KRAS mutation status with other clinical features are very valuable for understanding the nature of the disease and also planning of treatment strategies. In this

study, the KRAS mutation frequency in colonic type adenocarcinoma is compared with relevant clinical parameters. KRAS mutations subject to this study leads the constitutive activation of RAS signaling pathway. This activation of RAS pathway causes anti-EGFR monoclonal antibody treatments to be ineffective. Analysis of KRAS activating mutations has a predictive and prognostic value in identifying tumors that may confer resistance to treatment.

In our study, we have demonstrated that KRAS mutation status, age distributions, mutation type and codon distributions on our cohort composed of Turkish patients are in accordance with the literature. Moreover, we have demonstrated that there is statistically significant correlation between grade and KRAS mutation status where well differentiated tumors are associated with lower KRAS mutation rate when Type 1 error value is taken as 0,1.

If our study is conducted with a larger cohort, the comparison of the KRAS mutations may have significant correlations with other clinical features. Reflections of aforementioned information related to patients must be further investigated with clinical studies with larger cohorts. We aim to contribute the accumulation of knowledge related to tumorigenesis and cancer treatment by comparing KRAS mutations status and type with other clinicopathologic parameters with this study.

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Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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