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Clinicopathological Characteristics and KRAS-mutation Incidence in Gastric Carcinomas

Mide Kanserlerinin Klinikopatolojik Özellikleri ve KRAS-mutasyon İnsidansı

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Abstract

Objectives: This study aimed to determine the frequency of KRAS mutations in patients with gastric adenocarcinoma (GAC) in Hatay Province and the relationship of these mutations with some pathological and clinical parameters, and to guide the diagnosis and treatment planning of patients.

Material and Method: Formalin-fixed, paraffin-embedded and histologically confirmed samples were used in the assessment of KRAS mutation. The sections were taken from the archive tissue samples of each case. Real-Time Polymerase Chain Reaction (RT-PCR) system was used to identify the mutations of codons 12 and 13 (exon 2) of the RAS gene. The mutations GLY12ALA (G12A), GLY12ASP (G12D), GLY12ARG (G12R), GLY12CYS (G12C), GLY12SER (G12S), GLY12VAL (G12V) and GLY13ASP (G13D) were investigated.

Results: The KRAS mutation rate was 2% and only G12D substitution was detected. In this case, the tumor was located in the small curvature. No statistical comparison could be carried out between KRAS mutation and clinicopathological factors due to the small number of the mutated cases. Tumor differentiation was found significantly different from WHO-2010 typing and primary tumor staging.

Conclusions: We have found the incidence of KRAS mutation to be 2%. Even though, KRAS mutation in GAC alone is not a prognostic or predictive marker, subtype-specific analysis can provide data that may affect the diagnosis, management and treatment of the disease.

Keywords: KRAS, gastric carcinoma, G12D, mutation

Öz

Amaç: Bu çalışmanın amacı, Hatay ilinde mide adenokarsinomlu (GAC) hastalarda KRAS mutasyonlarının sıklığını, bu mutasyonun bazı patolojik ve klinik parametrelerle ilişkisini belirlemek, hastaların tanı ve tedavi planlamasına rehberlik etmektir.

Gereç ve Yöntem: KRAS mutasyonunun değerlendirilmesinde formalinle fikse, parafine gömülmüş ve histolojik olarak tanıları doğrulanmış örnekler kullanıldı. Her vakanın arşiv doku örneklerinden kesitler alındı. RAS geninin 12 ve 13 kodonlarının (ekson 2) mutasyonlarını belirlemek için Gerçek Zamanlı Polimeraz Zincir Reaksiyonu (RT-PCR) sistemi kullanıldı. GLY12ALA (G12A), GLY12ASP (G12D), GLY12ARG (G12R), GLY12CYS (G12C), GLY12SER (G12S), GLY12VAL (G12V), GLY13ASP (G13D) mutasyonları çalışıldı.

Bulgular: KRAS mutasyon oranı %2 idi ve sadece G12D tespit edildi. Bu olguda, tümör küçük kurvatur yerleşimliydi. Mutasyonlu olgu sayısı az olduğundan KRAS mutasyonu ile klinikopatolojik faktörler arasında istatistiksel karşılaştırma yapılamadı. Tümör diferansiyasyonu ile WHO-2010 tiplemesi ve primer tümör evresi arasında anlamlı bir fark bulundu.

Sonuç: KRAS mutasyonu insidansını %2 olarak bulduk. GAC'deki KRAS mutasyonu tek başına prognostik veya prediktif bir belirteç olmasa da, alt tipe özgü analiz, hastalığın tanısını, yönetimini ve tedavisini etkileyebilecek veriler sağlayabilir.

Anahtar Kelimeler: KRAS, mide kanseri, G12D, mutasyon

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INTRODUCTION

Gastric cancers (GCs) are the 5th most common cancer type in the world and the 4th most common in males as well as being the 3rd most frequent cause of cancer-related deaths following lung and liver cancers.[1] As a general information, GCs are 2-fold more frequently seen in males than females. The current prevalence of GCs, molecular and geographical variants, variations in molecular levels, the complexity of treatment management and poor prognosis still pose a challenge for health professionals.[2] The GCs are mostly gastric adenocarcinoma (GAC), genetic and molecular pathways as well as factors such as Helicobacter pylori infection play an important role in its etiology.[3]

The differences between many countries and regions in terms of the frequency of GAC may be due to the genetic factors of the disease.[4] With increased identification of oncogenic mutations and cell signaling pathways, it has become possible to predict the subtypes of cancers and treatment success of targeted therapies has increased in various cancers.[5]

Rat sarcoma virus (RAS) proteins are located in the inner region of the cell membrane and mediate important cellular events such as cell growth, cell differentiation, cell skeleton organization, cell migration and signal transduction pathways that control membrane traffic, apoptosis, metastasis and angiogenesis. The RAS and phosphoinositide 3-kinase (PI3K) signaling pathway are the key signals that are activated in the different tumors.[4,6] Mutations in RAS and pathways mentioned previously have been detected in many types of cancer. Although the frequency of mutations in the RAS gene family varies depending on cancer type, approximately 30% of all cancers involve a point mutation in the RAS gene, and these mutations often occur in the Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) gene.[7,8]

The KRAS gene is located at 12p12.1 and consists of 6 exons. The KRAS protein, a small GTPase, consists of 188-189 amino acids. Two mRNA isoforms, KRAS4A and KRAS4B emerge as a result of alternative splicing occurring in the KRAS gene. The KRAS mutation status is important in predicting treatment efficacy of drugs such as cetuximab in targeted therapy. KRAS is involved in the epidermal growth factor receptor (EGFR) pathway. These proteins often mediate the transmission of proliferative signals through the upstream of receptor tyrosine kinases and downstream cascades of protein kinases. Mutations in the KRAS gene induce uncontrolled activation of RAS protein.[9,10] As traditional knowledge, KRAS mutations are contradictory to EGFR mutations and can induce EGFR-RAS-RAF-MAP kinase steps independent of the EGFR mutation and inhibition of apoptosis. For this reason, KRAS proteins appear to be resistant to EGFR inhibitor drugs. However, KRAS mutations are known to be a negative predictor and the KRAS mutation status depends on the EGFR mutation state. Therefore, it is still debated whether EGFR inhibitors are useful for GAC.[4,11]

This study aimed to determine the frequency of KRAS mutations in patients with GAC in Hatay province and the relationship of these mutations with some pathological and clinical parameters, and to guide the diagnosis and treatment planning of patients.

MATERIAL AND METHOD

Data collection and ethical approval: The present study included 49 diagnosed as primary GAC between 2007 and 2016 at Hatay Mustafa Kemal University School of Medicine. The histopathological sections and clinical information of the cases were examined retrospectively. Age and gender of the patients, tumor diagnosis, WHO-2010 typing.[12] and primary tumor stage, grade of differentiation, lymphovascular invasion, lymph node involvement were recorded as the clinicopathological data. Tumor differentiation was evaluated as well, moderately or poorly differentiated according to WHO differentiation grading.[12] It was then grouped as high grade (moderately+poorly differentiation) and low grade (well-differentiation), and primary tumor stages were grouped as pT2 and pT3+pT4 for statistical analysis.

KRAS Mutation Analysis: Formalin-fixed, paraffin-embedded and histologically confirmed samples were used in the assessment of KRAS mutation. Three to four sections with 3-4 µm thickness were taken from the archive tissue samples of each case and transferred to sterile microcentrifuge tubes. Genomic DNA was extracted from the samples using DNA isolation kit (Qiagen, Hilden, Germany). Genomic DNA isolations were performed using these tissue samples. Real-Time Polymerase Chain Reaction (RT-PCR) system was performed to identify the mutations of codons 12 and 13 (exon 2) of the RAS gene. KRAS mutations were analyzed in the samples after DNA isolation.The commercially available KRAS RT-PCR kit (Qiagen, Hilden, Germany) was for this procedure. The detection of mutations with this kit consists of two stages; (i) evaluation of DNA quality, and (ii) RT-PCR reactions. The quality of the samples should be evaluated to use these samples for RT-PCR reactions, . The samples with appropriate DNA quality were used in the mutation detection stage. The mutations GLY12ALA (G12A), GLY12ASP (G12D), GLY12ARG (G12R), GLY12CYS (G12C), GLY12SER (G12S), GLY12VAL (G12V), GLY13ASP (G13D) were investigated using this KRAS RT-PCR kit.

The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of Hatay Mustafa Kemal University (Decision Number: 2017/154). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis

Statistical Analysis: SPSS Version 21.00 (NY, Armonk) was employed for statistical analysis. Kolmogorov Smirnov test was used to test normality of the distributions. Quantitative variables were expressed as mean and standard deviation

(SD) while categorical variables were expressed as number (n) and percentage (%). Chi-square test or Fisher's exact test was used to compare qualitative data. The differences between tumor differentiation groups regarding qualitative data were analyzed by Mann-Whitney U test. A p-value < 0.05 was considered statistically significant.

RESULTS

The clinicopathological characteristics of patients: The clinical and pathological characteristics of 49 participants were summarized in **Table 1**. The patient ages ranged between 30-90 years (min-max) while mean age was 62.37±16.79 years. The tumor diameter ranged between 14-105 mm (min-max) mm while mean tumor diameter was 61.85±24.31 mm. All the patients were diagnosed as GAC. According to WHO 2010 criteria, tubular GAC was found in majority of the patients (49%). Other subtypes were poorly cohesive (40.8%), mucinous (8.2%) and signet-ring cell (2.0%) carcinomas, respectively. According to assessment of primary tumor stage;, the majority of patients (69.4%) were pT4. Of the patients; 49.0%, 32.7% and 18.4% had poorly-differentiated, moderately-differentiated and well-differentiated tumors, respectively.

KRAS Mutation

The mutation rate of KRAS was 2% (1 in 49) and only one substitution, G12D, was detected. No statistical comparison could be carried out between KRAS mutation and clinicopathological factors due to the small number of the mutated cases.

Tumor Differentiation

Table 2 shows the comparison between tumor differentiation grades and some clinicopathological characteristics. In the comparison, Tumor differentiation was found significantly different from WHO-2010 typing and primary tumor staging. According to WHO-2010 typing, more than half of the tubular type cases (79.2%) were high grade. The number of the cases with signet-ring cell (n=1) and mucinous (n=4) type tumors was low for the evaluation .In primary tumor staging, the majority of patients (62.5%) in pT2 stage were low grade, whereas the majority of patients (90.2%) in pT3+pT4 stages were high grade.

DISCUSSION

The first report on KRAS mutations in GAC patients was published in 1986. Since then, many studies have investigated the status of KRAS mutations in GAC. Until recent times, the vast majority of studies have been conducted on Asian GAC patients. The largest Western study included 82 patients whereas while an Asian (China) study included 485 GAC patients.[5,13] However, nowadays, multicenter studies such as researches conducted on 1282 GAC patients in the largest have started to be published.[9]

Comparing the mutational pattern of GAC, The Cancer Genome Atlas (TCGA) study identified 25 significantly mutated genes in GAC including KRAS.[14] KRAS is a downstream effector of EGFR and activating mutation of KRAS is considered to stimulate the RAS/signaling pathway

Table 1. The clinical and pathological characteristics of 49 participants

Data		
Age (mean±SD)	62.37±16.79	
Tumor size (mean±SD)	61.85±24.31 mm	
	N	**%**
Gender		
Male	35	71.4
Female	14	28.6
Diagnosis		
Adenocarcinoma	49	100
WHO-2016 typing		
Tubular	24	49
Poorly cohesive	20	40.8
Signet-ring cell	1	2
Mucinous	4	8.2
T stage		
pT2	8	16.3
pT3	7	14.3
pT4	34	69.4
Tumor differentiation		
Poorly	24	49
Moderately	16	32.7
Well	9	18.4

SD: Standard Deviation

Table 2. Comparison between tumor differentiation and some clinicopathological characteristics

Category	Variable	Tumor Differentiation		p value
		High Grade	Low grade	
Gender				0.702
	Male	29 (82.9%)	6 (17.1%)	
	Female	11 (78.6%)	3 (21.4%)	
WHO-2016 typing				0.725
	Tubular	19 (79.2%)	5 (20.8%)	
	Others	21 (84.0%)	4 (16.0%)	
LVI				0.179
	Present	33 (86.8%)	5 (13.2%)	
	Absent	7 (63.6%)	4 (36.4%)	
LN Metastasis				0.259
	Present	27 (87.1%)	4 (12.9%)	
	Absent	13 (72.2%)	5 (27.8%)	
PSC				0.569
	Present	5 (100.0%)	0 (0.0%)	
	Absent	35 (79.55%)	9 (20.5%)	
DSC				1.00
	Present	3 (100.0%)	0 (0.0%)	
	Absent	37 (80.4%)	9 (19.6%)	
T stage				0.003*
	pT2	3 (37.5%)	5 (62.5%)	
	pT3+pT4	37 (90.2%)	4 (9.8%)	

LVI: Lymphovascular invasion, LN: Lymph node, PSC: Proximal surgical margin, DSC: Distal surgical margin

independently from EGFR activation. The recent studies suggest that KRAS activation and downstream signaling may affect the properties and functions of the tumor microenvironment.[9]

The status of KRAS mutation is important in predicting therapeutic efficacy of drugs such as cetuximab (anti-epidermal growth factor receptor) in targeted therapy. For example, KRAS is a crucial biological marker in predicting response to anti-EGFR treatment in colorectal cancers.[15]

In the traditional knowledge, GCs are 2-fold more frequently seen in males than females. Also Fu et al.[5] have found that the frequency of GAC males is 2-fold higher compared with females. In a multicenter study that involved 1282 GAC patients, the male/female ratio was 3/2.[9] In our study, the prevalence of men with GAC was more than 2-folds of that in female. This finding was partially consistent with literature data.[4,5]

The rate of KRAS mutation on 485 patients with GC (China) revealed that the rate of KRAS mutation was 4.1%.[5] In other studies, similar rates were detected in Japanese (4%-4.9%) and Chinese (4.5%) patients.[13,16,17] In a study carried out on 120 GAC patients in Iran, the KRAS mutation rates were 13.3% (codon 12) and 16.7% (codon 13).[4]

In a study performed on fresh frozen samples tissues of 595 GC patients from Italy and Singapore, KRAS mutation was reported to be 4%. The researchers also observed that KRAS mutations were more common in older patients.[18] In a multicenter study that involved 1282 GAC patients, the KRAS mutation rate was found to be 5%.[9] In a review of the literature, mean KRAS mutation rate in GAC patients was estimated to be 6.5%.[19]

In our study, KRAS mutation was detected in only one patient (G12D mutation), and its rate was 2%. Our findings were lower than the literature average. We consider that the small number of cases, racial differences in mutation rates, varying methodologies, and different analysis methods may be the factors leading to our findings.

Fu et al. have examined the relationship between KRAS mutation and some clinicopathological characteristics (gender, age, TNM stage, tumor location, and lymph node status) in GC patients. In their study, they have detected a significant correlation between KRAS G12D mutation and tumor location (p=0.020) and lymph node invasion (p=0.045). The researchers have determined that the G12D mutation was more concentrated in the upper and middle gastric locations and pNO type.[5]

Ayatollahi et al. have found that KRAS codon 12 mutation was related with tumor classification while KRAS codon 13 mutation was related with tumor site and tumor classification.[4] They have detected KRAS codon 12 and KRAS codon 13 mutations in half of the T1 cases and two-thirds of gastric fundus tumors, respectively. In another study, it was observed that GACs with KRAS mutations developed from the gastric antrum.[20] The mutational patterns of 77 genes were studied in 91 poorly cohesive GAC patients in South Korea, beside several gene mutations, KRAS mutations were significantly associated with tumor location (more commonly in upper 1/3 region), tumor depth (T1 versus T2+T3+T4), growth pattern of tumor and lymphovascular invasion-embolism.[21]

Hewit et al. have identified a significant relationship between KRAS mutation and tumor histology including 1282 GAC patients in countries such as Japan and England in their study. According to Lauren's classification, The highest (12%) and the lowest (2%) KRAS mutation rates were detected in mucinous and diffuse type GAC cases, respectively.[9] The prognostic significance of KRAS mutation in GAC is controversial. While no correlation was found between the existence of KRAS mutations and survival in GAC in some studies,[19] they were detected to be more associated with poor prognosis in the lung and colorectal cancers[22] whereas KRAS mutation has been determined to be associated with improved prognosis in ovarian cancer.[23]

Most of the KRAS-mutated cases were reported as moderately differentiated tumors by Ayatollahi et al.[4], Zhao et al.[20] reported KRAS-mutated cases as well-differentiated tumors. Ayatollahi et al.[4] observed that 50% of KRAS-mutated cases were T1 tumors. However, in other studies, no significant relationship was established between KRAS mutation and TNM stage.[24] In our study, there was only one case with mutation so no statistical comparison could be carried out between KRAS mutation and clinicopathological factors.

In our study, tumor differentiation was found significantly different from WHO-2010 typing and primary tumor staging. According to WHO-2016 typing, more than half of the tubular type cases (58.3%) were moderately-differentiated type. However, 90% of the poorly cohesive carcinomas were poorly-differentiated type. Besides, most of the tumors invading the serosa layer (pT4 tumor) were poorly (58.8%) and moderately (32.4%) differentiated GAC. The results obtained in the subserosa invasion (pT3 tumor) were similar to those reported in the serosa. However, the majority of the cases (62.5%) with muscularis propria invasion (pT2 tumor) were well-differentiated GAC. In primary tumor classification, the majority of patients (62%) were well-differentiated in pT2 stage whereas the majority of patients (58.8%) in pT4 were poorly differentiated.

Limitations

The small sampling size, inclusion of the cases only from a single institution (Hatay Mustafa Kemal University, Medical Faculty), investigation of only KRAS-gene types and inability to examine closely related genes (such as BRAF, PIK3CA) were the limitations of our study.

CONCLUSION

In our study, we found the incidence of KRAS mutation to be 2%. This result is different from Europe, Middle Eastern and the Far Eastern countries due to some factors limiting

our study as well as regional and racial variations. Although, KRAS mutation in GAC per se is not a prognostic or predictive marker, subtype-specific analyses may provide data that may affect the diagnosis, management and treatment planning of the disease. Besides, further comprehensive analyses and novel studies with larger sample sizes are needed for more precise outcomes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of Hatay Mustafa Kemal University (Decision No: 2017/154).

Informed Consent: Written consent was obtained from the families for their participation in the study.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of 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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