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The Relationship Between KRAS Mutation and ^{18}F-FDG Uptake Parameters in Colorectal Cancer

Kolorektal Karsinomda KRAS Mutasyonu ve ^{18}F-FDG Tutulum Parametreleri Arasındaki İlişki

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Abstract

Objective: Our purpose in this study was to evaluate whether Kirsten rat sarcoma viral oncogene (KRAS) exon-2 mutation affected ^{18}F-fluorodeoxyglucose (FDG) accumulation patterns, total lesion glycolysis and metabolic tumor volume in colorectal cancer.

Method: This retrospective study included 52 colorectal cancer patients. Dual-time ^{18}F-FDG positron emission tomography/computed tomography (PET/CT) parameters such as the maximum standardized uptake values (SUV_{max}), tumor-to-liver parenchyma SUV_{max} ratios (TLR), retention index (RI), metabolic tumor volumes (MTV), total lesion glycolysis (TLG) and glucose corrected-TLGs were measured.

Results: There were no statistical differences in PET/CT imaging parameters between mutated and wild-type colon cancer, but RI and RI (TLR) values were statically higher in wild-type than in mutated-type. KRAS exon-2 wild-type rectal cancer patients had low MTV (p=0.044). KRAS mutation status was correlated with MTV (r=-0.277, p=0.048). ROC curves analysis showed that MTV and MTV (%) predicted KRAS exon-2 mutation status accurately.

Conclusion: Although we did not find a relationship between KRAS exon-2 mutation status and increased ^{18}F-FDG uptake in both colon and rectal cancer patients in our study, KRAS exon-2 wild-type colon cancer patients showed interestingly increased uptake of ^{18}F-FDG in time. Even if we find a correlation between KRAS exon-2 mutation status and MTV, it was not very strong.

Keywords: Colorectal neoplasms, fluorodeoxyglucose F18, KRAS protein, human, positron emission tomography

Öz

Amaç: Bu çalışmadaki amacımız, kolorektal kanserde Kirsten sıçan sarkom viral onkogeni (KRAS) ekson-2 mutasyonunun ^{18}F-florodeoksiglukoz (FDG) tutulum paternlerini, toplam lezyon glikolizini ve metabolik tümör hacmini etkileyip etkilemediğini değerlendirmektir.

Yöntem: Bu retrospektif çalışmaya 52 kolorektal kanserli hasta dahil edildi. Maksimum standartlaştırılmış uptake değerleri (SUD_{maks}), tümör-karaciğer parankim SUV_{maks} oranları (TKO), retansiyon indeksi (RI), metabolik tümör hacimleri (MTV), toplam lezyon glikoliz (TLG) ve glukozla düzeltilmiş TLG'ler gibi çift zamanlı ^{18}F-FDG pozitron emisyon tomografi/bilgisayarlı tomografi (PET/BT) parametreleri ölçüldü.

Bulgular: Mutasyona uğramış ve vahşi tip kolon kanseri arasında PET/BT görüntüleme parametrelerinde istatistiksel fark yoktu, ancak RI ve RI (TKO) değerleri vahşi tipte mutasyona uğramış tipten istatiksel olarak daha yüksekti. KRAS ekson-2 vahşi tip rektum kanseri hastalarında düşük MTV vardı (p=0,044). KRAS mutasyon durumu MTV ile korele idi (r=-0,277, p=0,048). ROC eğrileri analizi, MTV ve MTV'nin (%) KRAS ekson-2 mutasyon durumunu doğru bir şekilde öngördüğünü gösterdi.

Sonuç: Çalışmamızda hem kolon hem de rektum kanserli hastalarda KRAS ekson-2 mutasyon durumu ile artmış ^{18}F-FDG tutulumu arasında bir ilişki bulamasak da, KRAS ekson-2 vahşi tip kolon kanserli hastalarda ilginç bir şekilde zamanla artan ^{18}F-FDG tutulumu görülmüştür. KRAS ekson-2 mutasyon durumu ile MTV arasında bir korelasyon bulsak bile, bu çok güçlü değildi.

Anahtar kelimeler: Florodeoksiglukoz F18, insan, KRAS proteini, kolorektal neoplazmalar, pozitron emisyon tomografi

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Introduction

The colorectal cancer (CRC) is one of the most common cancer types worldwide (1). By rising developments in diagnostic imaging modalities and optimization of surgical, neoadjuvant and palliative therapies, the mortality rate of CRC has decreased by more than 20% in the last 10 years. Pathogenesis of CRC is still not clear. Initially, it was thought that genetic mutations and chronic inflammation played the key role in its pathogenesis. Approximately 35-40% of CRC exhibits a mutation in the V-KI-RAS2 Kirsten rat sarcoma viral oncogene (KRAS) that involved codons 12 and 13 in more than 90% of cases. Although testing for mutations in KRAS exon 2 containing codons 12 and 13 was recommended previously, the current guidelines recommend to analyze not only KRAS exon-2 but also KRAS exons-3, containing codons 59 and 61 and exon-4, containing codons 117 and 146, NRAS exons-2, containing codons 12 and 13, exons-3, containing codons 59 and 61, and exons-4, containing codons 117 and 146 (2). Clinical significance of RAS mutation is associated with resistance to drugs targeting the epidermal growth factor receptor (EGFR), which is linked to cell survival, motility, proliferation, angiogenesis and metastasis. Currently, anti-EGFR drugs are recommended for wild-type KRAS metastatic CRC patients.

Molecular imaging has gained wide acceptance in many clinical oncology practices as a less invasive diagnostic technique. For this purpose, imaging is performed with positron emission tomography combined with computed tomography (PET/CT), in which tumor-seeking agents are used. It allows molecular and morphologic evaluation at the same time and displays a powerful tool within one imaging modality for whole body staging, restaging, and evaluation of therapy. The radiopharmaceutical commonly used in clinical routine for PET/CT scans is ^{18}F-fluorodeoxyglucose (FDG), which is an analog of glucose. Thus, glucose metabolism is measured *in vivo* by uptake of ^{18}F-FDG. Likewise, in glucose metabolism, it is taken into the cell via glucose transporters (GLUT) and then phosphorylated to FDG-6-phosphate by hexokinases and trapped inside the cell. Using delayed or dual-time PET/CT scans in molecular imaging has been suggested by some researchers because uptake of ^{18}F-FDG uptake increases markedly in malignant cells over time. Semi-quantitative parameters calculated on ^{18}F-FDG PET/CT scans are standardized uptake value (SUV), reflecting ^{18}F-FDG accumulation, metabolic tumor volume (MTV), and total lesion glycolysis (TLG). There are some human studies demonstrating the relationship between SUV and KRAS mutational status (3-9). However, in most of these studies, FDG accumulation pattern in dual-time imaging, glucose corrected-TLG (cor-TLG) and also MTV and TLG were not studied. The aim in this study was to analyze whether KRAS exon-2 mutation affected ^{18}F-FDG accumulation patterns in dual-time PET/CT imaging, MTV, TLG, and cor-TLG in CRC.

Materials and Methods

We retrospectively reviewed the medical records of all CRC patients who underwent ^{18}F-FDG PET/CT scan as a part of a staging work-up before treatment at our institution between February 2010 and August 2015. We enrolled 52 patients (mean age: 59.65±14.053 years, 23 female and 29 male) with CRC who had KRAS exon-2 gene mutation analysis and underwent preoperative ^{18}F-FDG PET/CT scan before resection or neoadjuvant treatment. CRC was diagnosed by colonoscopical biopsy in all patients before ^{18}F-FDG PET/CT imaging. Local ethics committee approved this retrospective study. Due to the retrospective design of this study, the requirement for informed consent was not deemed necessary.

PET/CT scans were achieved with a Gemini GXL PET/CT scanner (Philips Healthcare, Cleveland, Ohio, USA) after intravenous 354.31±56.5 MBq (9.57±1.53 mCi) ^{18}F-FDG injection when patients were fasted for at least six hours prior to injection. Also, blood glucose levels were checked just prior to injection. An oral contrast agent was used for each patient. After injection, all patients were rested in a quiet room for a good FDG distribution during the waiting period. They emptied their bladder before scanning. Whole body ^{18}F-FDG PET/CT imaging at 1 hour was acquired from the skull base to the mid thighs in supine position. A CT image was achieved firstly from the integrated PET/CT scanner with the use of a standardized protocol. This involved a section thickness of 3.3 mm, 120 kV, automatically calculated mA·s for the patient's weight, a tube rotation time of 0.75 s per rotation, and a pitch of 0.85. Afterwards, PET images were acquired immediately and reconstructed using CT data for attenuation correction with iterative reconstruction. In 37 (71.15%) of the patients, delayed imaging was performed to better localize suspicious or primary lesions after 104.31±34.11 minutes from whole body scan.

For quantitative assessment, nuclear medicine specialist with ten years of experience evaluated the images of ^{18}F-FDG PET/CT with visual inspection in transaxial, coronal and sagittal planes using a commercial workstation

(IntelliSpace Portal; Philips Healthcare, USA). The regions of interest were drawn over the tumor. The maximum SUV (SUV_{max}) at whole body image (SUV1) and delayed spot image (SUV2) were automatically calculated with polygonal free-hand regions of interest in all patients. Furthermore, SUV_{max} was measured from the normal liver parenchyma with circular regions of interest at dual-time ($SUV1_{liver}$ and $SUV2_{liver}$). Non-tumor regions of interest were drawn larger than 1 cm. For tumor-to-liver SUV_{max} ratio calculation (TLR), SUV_{max} of primary tumor was divided to liver SUV_{max} as follows:

$TLR1 = SUV1/SUV1_{liver}$

$TLR2 = SUV2/SUV2_{liver}$

The retention index (RI) from SUV_{max} and TLR values, obtained dual-time images, was calculated via the formula below:

$RI = (SUV2 - SUV1) \times 100/(SUV1)$

$RI\ (TLR) = (TLR2-TLR1) \times 100/(TLR1)$

Additionally, MTV was automatically calculated from primary lesions on whole body PET/CT images with two different methods (Figure 1). The drawn regions of interest that protrudes the lesion in each section were checked. The first method described as MTV was that the contouring margins around the tumor were defined using a fixed SUV_{max} cut-off level, 2.5 or greater. Another method described as MTV (%) was that contouring margins were defined using a relative threshold with 40% or greater of SUV_{max}.

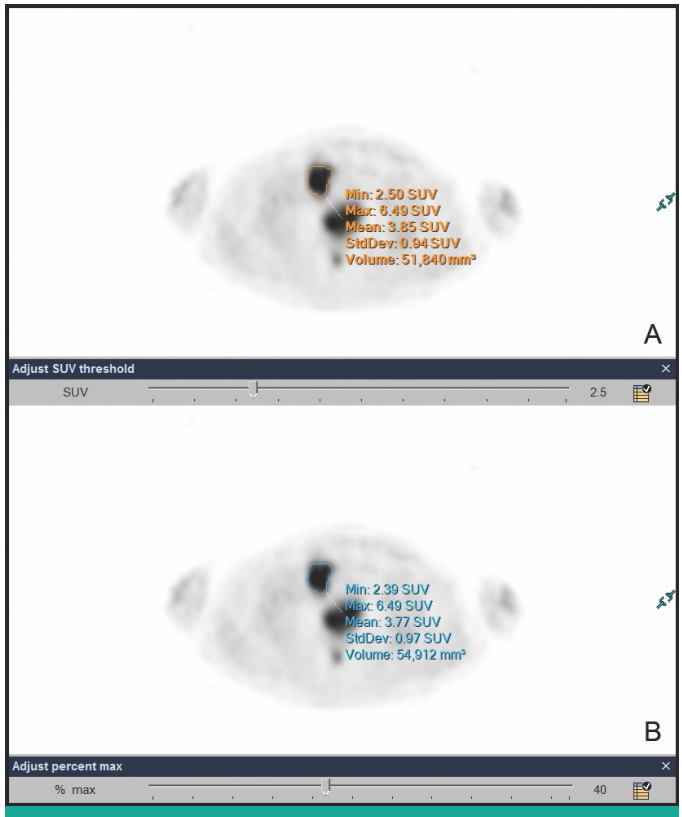

Figure 1. Calculation of metabolic tumor volume. The contouring margins around the tumor were defined using A) A fixed SUV cut-off level, 2.5 or greater and B) A relative threshold with 40% or greater of SUV_{max}

TLG values, considering both the metabolic activity and tumor burden, were calculated from primary lesion mean SUVs (SUV_{mean}) and MTVs (10) with this formula;

$TLG = SUV_{mean} \times MTV$.

In addition, SUV_{mean} corrected for the blood glucose level was calculated as (SUV_{mean}) × (blood glucose level)/100 (11), and then glucose corrected TLGs (cor-TLG) were also calculated from attenuation-corrected ^{18}F-FDG PET/CT images. Furthermore, values at two different methods such as TLG, TLG (%), cor-TLG, and cor-TLG (%) were defined depending on different MTV measurement methods, which is one parameter in TLG formula.

In 38 of 52 (73.1%) patients with metastasis at the diagnosis, KRAS gene mutation testing was conducted immediately. In the remaining 14 (26.9%), KRAS mutation gene testing was studied after detecting metastasis during follow-up. For KRAS mutation analysis, genomic deoxyribonucleic acid was extracted from formalin-fixed paraffin-embedded tumor tissue sections by using microdissection method. Pathologists experienced in gastrointestinal tumors, utilizing real-time polymerase chain reaction and pyrosequencing method, analyzed for the mutations specific for codons 12, 13, 61, and 146 of *KRAS* gene, and only exon-2 mutations were included in this study. Also, NRAS mutation status was not studied in all patients.

Statistical Analysis

Statistical calculations were carried out using the NCSS Statistical Software version 2007 (Kaysville, UT, USA). Quantitative parameters were analyzed by mean and standard deviation. Qualitative parameters were analyzed by percentage and frequencies. In case, the KRAS mutation status groups were classified as KRAS exon-2 mutated or KRAS exon-2 wild-type. The Mann-Whitney U test for variables was used. The Spearman correlation test among all parameters was performed. The analyses to compare the predictive ability were tested by receiver operating characteristic curve analysis. Values of p less than 0.05 were considered as significant.

Results

Thirty-one (59.6%) of the 52 patients had colon and 21 (40.4%) had rectal cancer. KRAS mutation status was KRAS exon-2 mutated in 14 (26.9%; 11 colon and 3 rectal cancer) patients and KRAS exon-2 wild-type in 38 (73.1%; 20 colon and 18 rectal cancer) patients. According to the KRAS status of patients, their descriptive statistics are presented in Table 1.

In ^{18}F-FDG PET/CT evaluations, the metastasis was detected in the liver in 30 patients, at the non-regional lymphatic station in the abdomen in 6 patients, and in the lung in 5 patients. 3 of patients had bone metastasis and 3 of them had peritoneal implants. The mean blood glucose level during ^{18}F-FDG injections was 107.38±19.748 mg/dL. Although there were no statistical differences in whole body PET/CT imaging parameters between mutated and wild-type colon cancer, RI and RI (TLR) values, reflecting gradually increasing glucose uptake, were higher in wild-type colon cancer than mutated patients (Figure 2). In rectal cancer, KRAS exon-2 wild-type patients had low MTV than mutated cases (Table 2).

Table 1. Descriptive statistics of patients

Characteristics	KRAS exon-2 mutated	KRAS exon-2 wild-type
n	14	38
Age (years)		
Mean ± SD*	61.5±12.72	58.97±14.62
Sex		
Female	8 (57.1%)	15 (39.5%)
Male	6 (42.9%)	23 (60.5%)
Blood glucose		
Mean ± SD*	109.43±25.157	106.63±17.692
CEA		
<5.0	2 (14.3%)	12 (31.6%)
≥5.0	12 (85.7%)	26 (68.4%)
Tumor localization		
Colon	11 (78.6%)	20 (52.6%)
Rectum	3 (21.4%)	18 (47.4%)
Metastases at diagnosis		
Yes	10 (71.4%)	28 (73.7%)
No	4 (28.6%)	10 (26.3%)
Tumor type		
Adenocarcinoma	12 (85.7%)	32 (84.2%)
Adenocarcinoma with mucinous differentiation	1 (7.1%)	4 (10.5%)
Signet ring cell adenocarcinoma	1(7.1%)	2 (5.3%)

* p-value>0.05, SD: Standard deviation, KRAS: Kirsten rat sarcoma viral oncogene

In correlation analyses, KRAS exon-2 mutation status was only correlated with MTV (%) (r=-0.277, p=0.048). SUV1 and SUV2 were strongly correlated with MTVs, TLGs, and cor-TLGs (Table 3 and Figure 3). The strong positive correlations were found between MTVs and TLGs because of the TLG derived from MTV. When receiver operating characteristic curves were analyzed to compare the efficacy of various parameters, the results showed that MTV and MTV (%) most accurately predicted the KRAS exon-2 mutated state (Figure 4). The areas under the curve were 0.722 [95% confidence interval (CI)=0.394-1] and 0.806 [95% CI=0.537-1], respectively. We then sought to determine optimal cut-off to distinguish between KRAS exon-2 mutated and wild-type patients. Receiver operating characteristic curve analysis showed cut-off value of 62.144 mm^3 for MTV with 63.64% sensitivity and 69.23% specificity (positive predictive value=46.7%, negative predictive value=81.8%, likelihood ratio=2.07). The cut-off value for MTV (%) was 31.616 mm^3 with 85.71% sensitivity and 60.53% specificity (positive predictive value=44.4%, negative predictive value=92%, likelihood ratio=2.17).

Discussion

The *RAS* gene family is a proto-oncogene that includes the oncogenes KRAS, HRAS, and NRAS. They have similar structure and function. When they become active, they begin to function as oncogenes and play an important role in the pathogenesis of cancer. RAS is involved in processes such as signal transduction, proliferation, mutation, adhesion, apoptosis and migration of cells. When RAS and RAS-associated proteins are increased, they often lead to the formation of cancers by increasing invasion and metastasis and reducing apoptosis (12). Many human malignancies, including lung cancer, pancreatic cancer, and particularly CRC, show (13,14). Overexpressions of KRAS mutations at codons 12, 13, 59, 61, 117, and 146 have been shown to induce RAS protein activation. The mutations of KRAS in CRC occur in codons 12 and 13 with approximately rate of 97%, while other mutations such as codons 61 and 146 are less frequent (15). KRAS exon-2 mutations are commonly observed in approximately 35-40% of patients with CRC, while the frequency is 10-15% for other RAS mutations (16). Clinical significance of KRAS mutations is due to its effect on treatment selection.

Uptake of ^{18}F-FDG into cancer cells is complex. It is affected by tumor-related factors such as histological type, differentiation, hypoxia, tumor size and nontumor-related factors like diabetes mellitus. This radiopharmaceutical

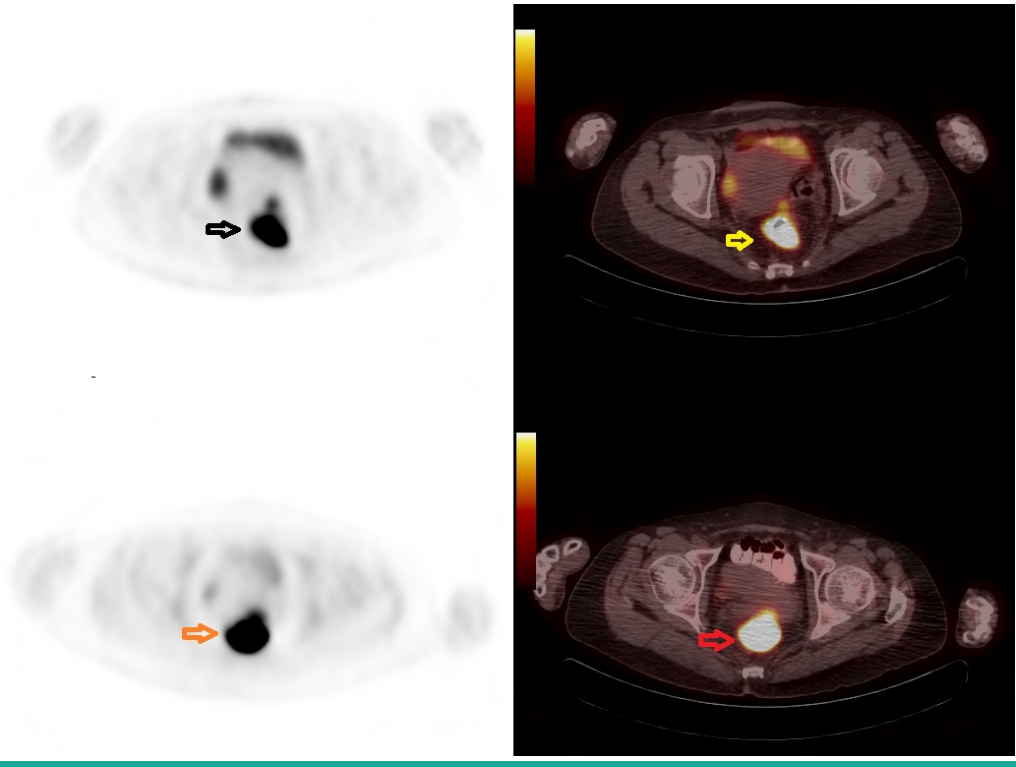

Figure 2. A 47-year-old female patient, KRAS wild type, rectal grade 2 adenocarcinoma. Early PET (black arrow) and fusion (yellow arrow) images in the upper row, late PET (orange arrow) and fusion (red arrow) images in the lower row. SUV_{max} values measured from the rectum were 28.5 and 37.1 for early and late images, respectively. In mutant type KRAS patients, there was no such difference for SUV_{max} values

PET: Positron emission tomography, KRAS: Kirsten rat sarcoma viral oncogene

Table 2. The Mann-Whitney U test for parameters of glucose metabolism from ^{18}F-FDG PET/CT scans according to KRAS exon-2 mutation status

	Colon cancer			Rectal cancer		
	Mutated	Wild		Mutated	Wild	
	Mean ± SD (n)	Mean ± SD (n)	p	Mean ± SD (n)	Mean ± SD (n)	p
Whole body imaging parameters						
SUV1	12.90±7.18 (11)	13.03±7.85 (20)	>0.05	17.18±6.76 (3)	14.17±6.07 (18)	>0.05
TLR1	6.05±3.31 (11)	5.51±3.303 (19)	>0.05	11.51±10.24 (3)	6.34±2.716 (18)	>0.05
MTV (mm^3)	91.537±86.596 (11)	77.722±96.798 (20)	>0.05	111.467±33.166 (3)	56.825±58.990 (18)	0.044
TLG	589.18±555.11 (11)	519.27±868.36 (20)	>0.05	689.00±253.73 (3)	396.93±484.73 (18)	>0.05
cor-TLG	686.28±700.65 (11)	619.44±1177.70 (20)	>0.05	693.11±170.45 (3)	425.53±515.23 (18)	>0.05
MTV (%) (mm^3)	73.117±60.994 (11)	55.066±64.531 (20)	>0.05	57.493±17.615 (3)	41.407±42.203 (18)	>0.05
TLG (%)	524.07±469.37 (11)	411.60±650.26 (20)	>0.05	465.27±66.38 (3)	329.37±400.46 (18)	>0.05
cor-TLG (%)	595.89±546.52 (11)	488.86±877.54 (20)	>0.05	477.04±52.15 (3)	351.03±421.69 (18)	>0.05
Delayed spot imaging parameters						
SUV2	15.79±10.059 (8)	21.02±11.67 (14)	>0.05	N/A	18.08±7.53 (14)	N/A
TLR2	7.86±4.44 (8)	9.98±5.57 (13)	>0.05	N/A	8.98±4.02 (14)	N/A
RI	24.09±10.51 (8)	40.15±16.29 (14)	0.017	N/A	32.64±33.27 (14)	N/A
RI (TLR)	31.89±13.93 (8)	57.78±33.34 (13)	0.030	N/A	45.38±41.74 (14)	N/A

SD: Standard deviation, KRAS: Kirsten rat sarcoma viral oncogene, MTV: Metabolic tumor volumes, TLG: Total lesion glycolysis, FDG: Fluorodeoxyglucose, PET/CT: Positron emission tomography/computed tomography

Table 3. The Spearman correlation test of ^{18}F-FDG PET/CT parameters

		SUV1	SUV2	TLR1	TLR2
MTV	r	0.535	0.519	0.457	0.440
	p	0.000	0.001	0.001	0.007
TLG	r	0.665	0.625	0.570	0.521
	p	0.000	0.000	0.000	0.001
cor-TLG	r	0.665	0.633	0.561	0.522
	p	0.000	0.000	0.000	0.001
MTV (%)	r	0.321	0.292	0.273	0.214
	p	0.020	0.080	0.053	0.210
TLG (%)	r	0.591	0.550	0.498	0.451
	p	0.000	0.000	0.000	0.006
cor-TLG (%)	r	0.585	0.551	0.488	0.452
	p	0.000	0.000	0.000	0.006

r: Correlation coefficient, FDG: Fluorodeoxyglucose, PET/CT: Positron emission tomography/computed tomography, MTV: Metabolic tumor volumes, TLG: Total lesion glycolysis

is transported into the cell through GLUTs. On the other hand, uptake of ^{18}F-FDG can also be seen in inflammatory processes. Depending on the different levels and degrees of GLUT and hexokinase expressions, there are profile differences in inflammatory lesions and cancer. For this purpose, dual-time imaging can be used for the differential diagnosis. Several studies have recently reported that the most essential factor for ^{18}F-FDG uptake in CRC is increased GLUT1 expression (5,17). Accumulation of ^{18}F-FDG in colon carcinoma can predict the underlying tumor biology in terms of forecasting of malignancy potential and prognosis (17). Furthermore, colon may occasionally demonstrate high ^{18}F-FDG uptake. Moreover, dual-time imaging is also useful in distinguishing tumors from normal tissues seen with high ^{18}F-FDG uptake. However, focal intense hypermetabolism is highly suggestive in neoplasm (18). Due to the fact that subcentimetric lesions may cause false negative results in ^{18}F-FDG PET/CT scanning, we perform

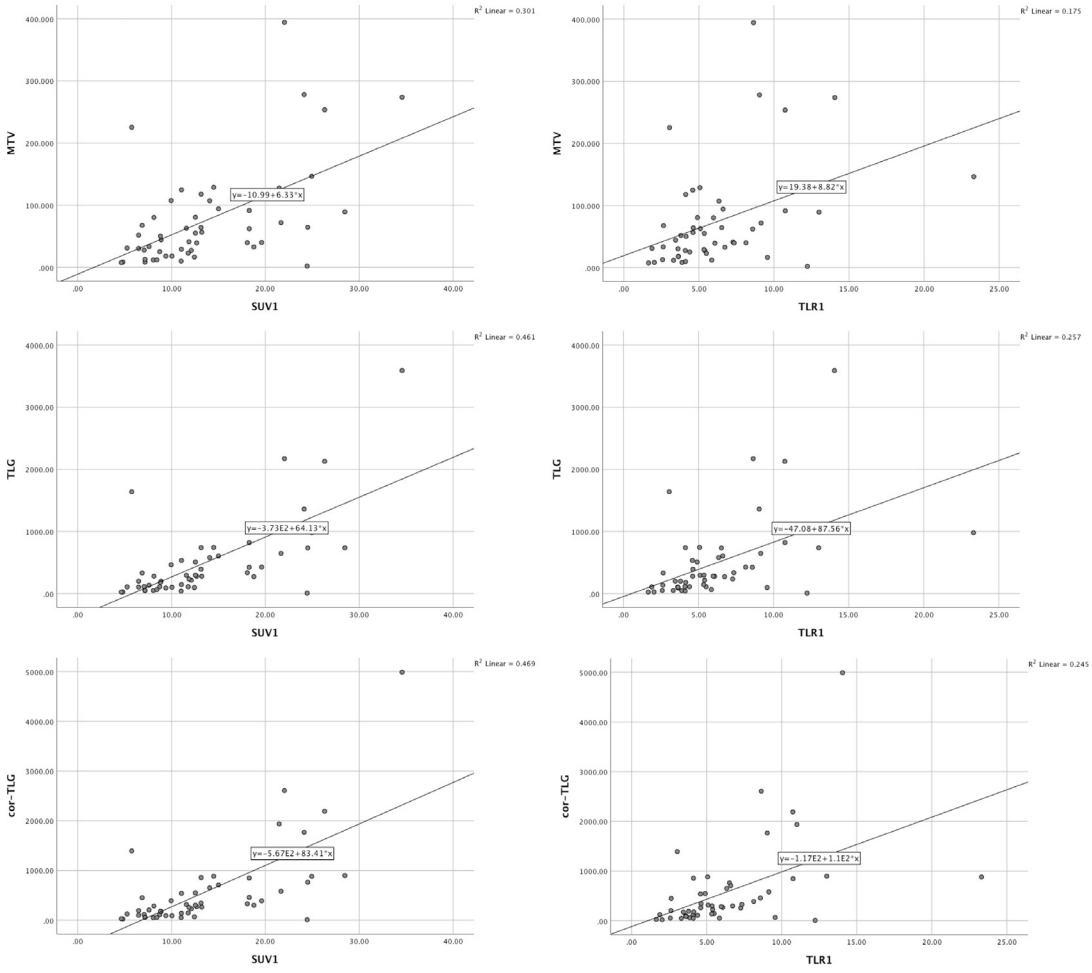

Figure 3. Correlation graphs of ^{18}F-FDG parameters
FDG: Fluorodeoxyglucose

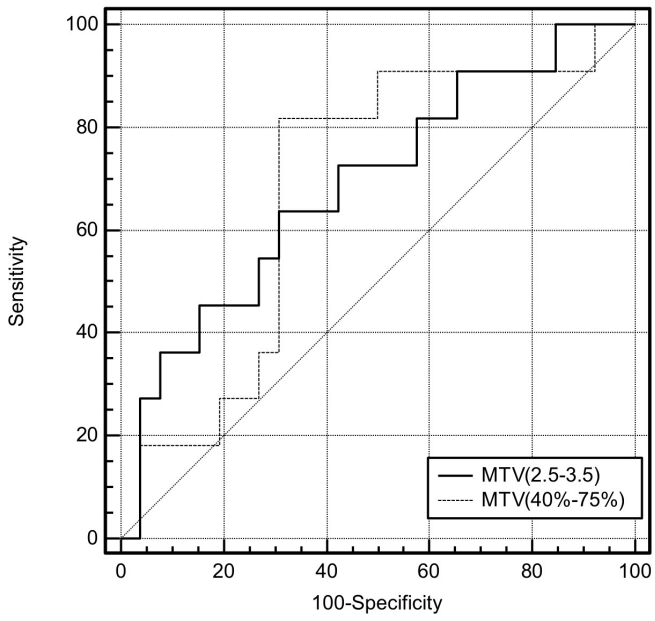

Figure 4. Receiver operating characteristic curve analysis of different MTV measurement methods to compare their diagnostic performance in detecting KRAS mutation status

KRAS: Kirsten rat sarcoma viral oncogene, MTV: Metabolic tumor volumes

^{18}F-FDG PET/CT imaging for patients with lesion size larger than 1 cm to avoid partial volume effect in our institution.

Interestingly, some studies have found that mutant KRAS or BRAF alleles have always higher GLUT1 transcript expression times compared to wild type, ranging from 3- to 22- fold (5,19,20). These CRC cells are thus able to survive under low glucose conditions with increased GLUT1 expression (20). At this point, more ^{18}F-FDG uptakes can be expected in patients with KRAS-mutant. Some studies in the CRC have examined this hypothesis in the literature (3-9). Firstly, Kawada et al. (5) investigated the relationship between ^{18}F-FDG accumulations of KRAS/BRAF mutations in 51 CRC patients. They calculated SUV_{max} for primary tumor and TLR, as well as evaluated GLUT1 and hexokinase type-II levels. They found that both SUV_{max} and TLR were significantly higher in the mutant group. Afterwards, Miles et al. (9) found that KRAS mutants with high SUV_{max} had a significantly increased likelihood of expressing hypoxia-inducible factor-1, while KRAS mutants with low SUV_{max} expressed minichromosome maintenance protein 2 in 33 CRC patients. In a study comparing KRAS exon-2 mutational status and SUV_{max} at the metastatic lesions of 44 CRC patients, they expressed no statistically significant correlation between SUV_{max} and KRAS mutation status in liver metastasis (7). A recent study by Chen et al. (3) in 121 CRC patients revealed that KRAS-mutated tumor exhibited higher SUV_{max}. In addition, researchers found that SUV_{max} and PET-based maximal tumor width with a 40% threshold of SUV_{max} were two predictors of KRAS mutations. In this study, MTVs and TLGs with different thresholds, such as 2.5 or 3.0 SUV_{max}, and volume greater than 20%, 30%, 40%, 50% of SUV_{max}, were calculated and no significant difference was found for *KRAS* gene mutation. In another study, researchers demonstrated that GLUT1 protein expression was about 2.5-3.0-fold in higher KRAS mutant in paired isogenic human CRC cell lines, and hexokinase 2 protein expression was also dramatically higher in the mutant KRAS cells than in wild-type cells. Under hypoxic situations, the researchers showed that KRAS mutated CRC cells show increased ^{18}F-FDG accumulations depending on the hypoxia-inducible factor 1κ pathway, whereas the enhanced uptake of ^{18}F-FDG in hypoxic wild-type CRC cells was independent of the hypoxia-inducible factor 1κ pathway (19). In a recent study, Chen et al. (4) found that SUV_{max}, various thresholds of MTV, TLG and tumor width on PET/CT yielded similar results for KRAS in 103 CRC patients as in other studies. In a second study of Kawada et al. (6) analyzing 55 tumors, they found no significant relationship between SUV_{max} and KRAS status including tumor size below 10 mm. Excluding cases with a small tumor size to minimize the bias of the partial volume effect, the researchers found higher SUV_{max} values in mutant KRAS patients than wild-type with 71.4% accuracy when the SUV_{max} cut-off value was 6.0. In a recently published study involving 179 CRC patients, researchers examined the value of PET/CT parameters such as SUV^{max}, SUV_{peak}, MTV, and TLG for the prediction of KRAS mutation and investigated their variability depending on C-reactive protein levels (8). They found higher accumulations of ^{18}F-FDG in CRC patients with KRAS mutations, and SUV_{max} and SUV_{peak} were independent predictors of KRAS mutation with positive LN metastasis. Also, the association changed with higher CRP levels.

In this study, we hypothesized that KRAS mutations would increase ^{18}F-FDG uptake especially in SUV2 and RI in dual-time imaging, and also affect the TLGs in CRC. With this aspect, our study is the first to evaluate delayed imaging findings and also to correct glucose-TLG values. We did not find a relationship between KRAS status and SUV_{max} of the primary lesion. This seems to be contrary to other publications in literature. However, in a meta-analysis, ^{18}F-FDG PET was shown to have low sensitivity and specificity for the prediction of KRAS mutation in CRC patients (21). On the other hand, we found higher levels

for RI and RI (TLR) parameters obtained from dual-time imaging in KRAS exon-2 wild-type colon cancer patients. However, we could not determine a cut-off value for RI and RI(TLR) in this study. Low MTV level was found in KRAS exon-2 wild-type rectal cancers. Beside TLGs, neither difference nor relationship was detected for cor-TLGs. The reason of discrepancy with literature for SUV_{max} was thought to be the performance of KRAS mutational analysis not only on the pathology specimens but also on samples of biopsy in our study as possible causes of discordance. In our study, KRAS mutation analysis was performed only in 73.1% of patients at the time of diagnosis. The remaining patients were examined for their metastatic lesions during their follow-up. Therefore, the KRAS mutation analyses of the patients were not homogeneous in terms of the samples examined. For this reason, the relationship between ^{18}F-FDG parameters according to KRAS sampling method was not evaluated in this study. However, Baas et al. (22) showed that tumor tissue had a high agreement of over 97% from endoscopic biopsies and matched resected specimens in the determination of RAS mutation status. Beside this, in different anatomical regions of the same patient, as an indicator of cancer, heterogeneity of KRAS mutational status can be determined as a molecular discordance (23-25). In addition, poor DNA quality in the tissue sample is seen in approximately 6% and 9% of cases, as a defect in determining KRAS mutation status (26).

Study Limitations

There are several limitations in our study. First of all, the greatest bias of this work derives from the retrospective nature of the study itself. Also, the number of patients was few in this study. The effects on ^{18}F-FDG accumulations of mutations of exon-3, 4 and also *NRAS* gene could not be assessed. While KRAS exon-2 mutation status was studied following tumor resection in a minority of patients, it was observed from biopsy specimen in others. Finally, the analysis of the mutational status of all patients was performed in a different laboratory.

Conclusion

As a result, although we did not find a relationship in our study between KRAS exon-2 mutation status and increased ^{18}F-FDG uptake in both colon and rectal cancer patients, which was previously expressed in literature, KRAS exon-2 wild-type colon cancer patients showed interestingly increased uptake of ^{18}F-FDG in time. Even if we were to find a correlation between KRAS exon-2 mutation status and MTV, it would not be very strong.. Therefore, we believe that further studies that examine subgroups for mutations and larger series are needed to clarify our hypothesis.

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Ethics

Ethics Committee Approval: This study was approved by Non-invasive Clinical Research Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (GOKAEK/2016-432).

Informed Consent: Due to the retrospective design of this study, the requirement for informed consent was not deemed necessary.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Conception/Design of Study: A.Ö., S.M., E.N., Data Acquisition: A.Ö., M.T., T.V., E.G., A.K.A, Data Analysis or Interpretation: A.Ö., A.K.A., F.Ç., Drafting Manuscript:A.Ö., M.T., T.V., E.G., Critical Revision of Manuscript: S.M., E.N., F.Ç., A.K.A., Final Approval and Accountability: A.Ö, S.M., E.N., A.K.A., M.T., T.V., E.G., F.Ç., Technical or Material Support: A.K.A., M.T., T.V., E.G., Supervision: A.Ö, S.M., E.N., F.Ç.

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