The value of ^{18}FDG PET/CT parameters, hematological parameters and tumor markers in predicting KRAS oncogene mutation in colorectal cancer

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Abstract

Objective: In this study we investigated the predictive value of maximum standardized uptake value (SUVmax), metabolic tumor volume (MTV), total lesion glycolysis (TLG), neutrophils/lymphocytes ratio (NLR), platelets/lymphocytes ratio (PLR), carcinoembryonic antigen (CEA) and carbohydrate antigen (CA 19-9) in the prediction of KRAS gene mutation which plays an important role in the choice of treatment in colorectal cancer patients. **Subjects and Methods:** A total of 55 cases with untreated colorectal cancer who had undergone both PET/CT examinations for initial staging and also mutation analysis of KRAS oncogene were studied. Fluorine-18-FDG PET/CT parameters (SUVmax, MTV, TLG), hematological parameters (NLR, PLR), and tumor markers (CEA, CA 19-9) were recorded and the relationship between these parameters and KRAS oncogene mutation was evaluated using receiver operating characteristics (ROC) analysis and multiple logistic regression analysis. **Results:** In 20 cases mutations in the KRAS gene were detected, while in 35 cases mutations were not observed (wild-type). ROC analysis revealed that SUVmax, MTV, TLG, NLR, PLR, and CA 19-9 could not predict mutations in KRAS oncogene (P=0.600, 0.263, 0.214, 0.057, 0.104, 0.189, respectively) although CEA value showed significant difference (P=0.031) but without high value of the area under the curve (0.676). Multivariate logistic regression analysis also did not show significant association between KRAS gene mutations and SUVmax, MTV, TLG, NLR, PLR, CEA, CA 19-9 values. **Conclusions:** We observed that in patients with colorectal cancers, we cannot predict KRAS gene mutations using PET/CT parameters (SUVmax, MTV, TLG), hematological parameters (NLR, PLR) or tumor marker CA 19-9. We detected a significant but not very strong association only between CEA and KRAS mutations.

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Introduction

Colorectal cancer with its subtypes is a frequently encountered disease worldwide [1, 2]. Determination of molecular markers (KRAS and BRAF oncogenes) have been used in the discrimination of cases of colorectal cancer, and its choice of treatment [3].

Mutations in KRAS oncogene are found in 40% of colorectal cancers. When molecular basis of agents targeted at epidermal growth factor receptor (EGFR) is taken into consideration, blockage of EGFR at the receptor level does not halt "downstream" signal activation in tumors with KRAS mutation and active Ras protein. Indeed, active Ras protein induces signal activation independent from EGFR. As a reflection of this information in clinical practice, it has been demonstrated that anti-EGFR antibody treatment with cetuximab and with panitumumab does not confer benefit in tumors with mutant KRAS gene [4-6]. Therefore, before application of anti-EGFR treatment, KRAS mutation analysis should be performed.

Fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) has been widely used in staging, in prognosis and in evaluation of treatment response in colorectal cancer. Standardized uptake value (SUV) is a semiquantitative parameter most frequently used in PET studies which demonstrate metabolic uptake of ^{18}F-FDG [7]. Standardized uptake value is used in the form of maximum SUV (SUVmax) and for mean SUV (SUVmean). Although SUVmax is widely used, it displays only SUV in the active region and does not provide information about dimensions of the tumor and tumor burden [8]. In recent years, new PET parameters are used which may overcome this handicap of SUVmax and give information about tumor metabolic activity, tumor burden, metabolic tumor volume (MTV) and total lesion glycolysis (TLG) [9, 10].

Though preoperative PET/CT imaging is very important in colorectal cancer patients, only a few studies have revealed the association between SUVmax, MTV and TLG derived from PET imaging and KRAS mutations. Kawada et al. (2012) observed higher SUVmax levels in colo-

rectal cancer patients with KRAS mutation [11]. On the contrary, Chen et al. (2015) demonstrated that SUVmax, MTV and TLG could not independently predict KRAS mutations [12].

The importance of inflammation stemming from tumor is already known. Some authors suggest that neutrophils/lymphocytes ratio (NLR) and platelets/lymphocytes ratio (PLR) derived from peripheral blood tests can be inflammation markers [13, 14] and are accepted as important prognostic indicators of various malignancies [15, 16]. In recent years NLR and PLR have been increasingly used in colorectal cancer patients. Pretreatment increase of these values is associated with poor prognosis [17, 18].

Tumor markers play important roles in the diagnosis, follow-up, treatment response and prediction of recurrence of some cancers. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) have been widely used in colorectal cancer patients [19].

In this study we investigated a number of factors: The predictive power of SUVmax, MTV and TLG values derived from ^{18}F-FDG PET/CT, the hematological parameters NLR and PLR and the tumor markers, CEA and CA 19-9 as predictors of KRAS gene mutations in order to evaluate each one of them in relation to KRAS gene mutations. In the literature, there are a few studies similar to ours which we mention in discussion.

Subjects and Methods

Study design

Fifty-five patients with untreated colorectal cancer diagnosed by histopathology, who had undergone PET/CT examination for initial staging and also mutation analysis of KRAS oncogene during October 2013 and December 2016 in Antalya Training and Research Hospital, Department of Nuclear Medicine were included in this retrospective study, after approval from the Ethics Committee of Antalya Training and Research Hospital. Patients had no other malignancy and were also tested for NLR, PLR and tumor markers CEA and CA 19-9 within 3 weeks before PET/CT examination.

PET/CT imaging

Each patient received intravenously 3.7MBq/kg ^{18}F-FDG after 6 hours of fasting when blood sugar levels were below 200mg/dL. After 45-60 minutes PET/CT scans were performed from vertex to upper femoral region using Philips Gemini TF 16 PET/CT scanner (CT 3mm section width, 110mAs, 120kV; for each bedside 3 minutes PET). The attenuated and corrected PET, CT and fusion PET/CT images were examined for the semiquantitative evaluations of SUVmax, SUVmean, MTV and TLG.

From the area of the highest activity of the primary tumor located in the colorectal region, a three-dimensional region of interest (ROI) was delineated to estimate the SUVmax value. Metabolic tumor volume was calculated using attenuation-corrected ^{18}F-FDG PET/CT images and defined as the sum of metabolic volumes of the primary tumor. During calculation of this volume drawings were made so as to contain all contours of the mass as measured from coronal, axial and sagittal planes. Forty percent of SUVmax was determined as the threshold and contours of the mass were drawn automatically (Extended Brilliance Workspace, Philips, USA). The MTV value obtained was recorded in cubic centimeters. Total lesion glycolysis value was also derived from attenuation-corrected ^{18}F-FDG PET/CT images. Forty percent of SUVmax of the primary tumor was determined as the threshold and contours of the mass were also drawn automatically. Mean standardized uptake value was determined within the contours of this area. Values of MTV and SUVmean were multiplied to obtain TLG value.

KRAS mutational analysis

QIAamp DNA FFPE tissue kit, buffer ATL, buffer AW1, buffer AW2, ATE buffer have been used (QIAamp DNA FFPE Tissue Handbook, June 2012, QUAGEN, Sample & Assays Technology).

As a first step, for DNA isolation, 8 serial 5μm-thick, and hematoxyline-eosin (HE) stained sections cut from blocks which contained ≥20% tumor cells fixed with formalin 10%, and embedded in paraffin were transferred into Eppendorf tubes.

For deparaffinization procedure QIAamp DNA FFPE tissue kit (QIAGEN, Hilden, Germany) was used. One milliliter xylene was added on samples taken into sterile 2mL centrifuge tubes, and vortexed. Then tubes were centrifuged at 8000 rpm for 2 minutes. Supernatant was withdrawn without touching the pellet. One milliliter 96%-100% ethanol was added on the pellet and centrifuged at maximum rpm for 2 minutes. The sample is kept under room temperature and exposed to air for 5 minutes till all alcohol was vaporized. Then 180μL buffer ATL and 25μL proteinase were added. The tubes were incubated in T-Shaker device till all the samples as lysed, for firstly for one hour at 56°C, and then at 70°C for another hour. Afterwards, buffer AL, ethanol, buffer AW1, buffer AW2 solutions were successively added on the lysed tissue samples, centrifuged at 6.000xg (9,000rpm) for 2 minutes. The tube containing spin column was discarded, and QIAampMinElute spin column was placed in a clean 2mL-collection tube which was centrifuged at 20,000xg (14,000 rpm) for 4 minutes. The sample obtained is eluted using 200 μL elution buffer (ATE buffer) in the QIAamp DNA FFPE tissue kit. In order to evaluate KRAS mutation in genomic DNA yield, we used control reaction mixture (CTRL) provided with therascreen KRAS RGQ polymerase chain reaction (PCR) kit. Therascreen KRAS RGQ PCR kit detects 7 potential mutations on Codon 12, and 13 localized in Exon2 on KRAS gen. Therascreen KRAS RGQ PCR kit, has been used with Rotor-Gene Q MDx device, after completion of the study results of analysis, and mutations have been displayed automatically by therascreen KRAS assay package software. Test results were displayed as "mutation positive", or "any mutation was not detected". Invalid results or control test failed results were described as "study control failed".

Statistical analyses

Data were analyzed using the IBM statistical package for social sciences v20 (SPSS Inc., Chicago, IL, USA). A normal distribution of the semi quantitative data was checked using Kolmogorov-Smirnov test. Parametric tests (independent-samples t-test) were applied to data of normal distribution and non-parametric tests (Mann–Whiney U-test) were applied to data of questionably normal distribution. The distribution of categorical variables in both groups was compared using Pearson chi-square test. To calculate correlation coefficients Pearson correlation test was used. Different predictive models were compared by ROC–area under the curve (AUC) statistics. Multiple logistic regression analysis was used to explore the determinants. Data were expressed as mean±SD or median (interquartile range), as appropriate. All differences associated with a chance probability of 0.05 or less were considered statistically significant.

Results

A total of 55 (female, n=16 and male, n=39) colorectal cancer patients who underwent ^{18}F-FDG PET/CT examination for primary staging and also mutation analysis of the KRAS gene were included in the study. Mean age of the patients was 59.20±14.28 years (age interval 26-86 yrs). In 20 cases mutations in the KRAS gene were detected, while in 35 cases mutations were not observed (wild-type). Primary tumors were observed in ascending colon (n=8), transverse colon (n=3), descending colon (n=3), sigmoid colon (n=5), rectosigmoid junction (n=4) and rectum (n=32). Mean SUVmax, SUVmean, MTV and TLG values for primary tumors and mean NLR, PLR, CEA and CA19-9 values of the patients are shown in Table 1.

Analyses using the independent-samples t-test and Mann Whitney U test showed that there were no significant differences between the wild-type and mutant KRAS groups in terms of age, SUVmax, SUVmean, MTV, TLG, CA19-9, NLR and PLR values (P>0.05). There was a significant difference only in CEA values (P<0.05), which was higher in the mutant group (Table 2). Furthermore, no significant difference was observed between males and females in terms of KRAS mutations according to the results of the Pearson chi-square test (P=0.614).

The predictive power of SUVmax, SUVmean, MTV and TLG values in the prediction of mutation in the KRAS oncogene was evaluated with ROC curve. Areas under ROC curve were 0.543±0.080 (P=0.600) for SUVmax, 0.543±0.080 (P=0.600) for SUV mean, 0.591±0.081 (P=0.263) for MTV and 0.601±0.077 (P=0.214) for TLG (Table 3).

The predictive power of hematological parameters as NLR and PLR values in the prediction of mutation in the KRAS oncogene were evaluated with ROC curve. Areas under ROC curve for NLR and PLR were (0.656±0.081) (P=0.057) and (0.633±0.079) (P=0.104) respectively (Table 3).

The predictive power of tumor markers as CEA and CA 19-9 values in the prediction of mutation in the KRAS oncogene

Table 1. *Mean, standard deviation, minimum and maximum values of SUVmax, MTV (cm3), TLG, NLR, PLR, CEA (ng/mL) and CA19-9 (U/mL).*

	N	Mean	Std. dev.	Min	Max
Age	55	59.20	14.28	26	86
SUVmax	55	16.64	8.49	4.76	49.02
SUVmean	55	8.65	4.73	2.77	27.15
MTV (cm^3)	55	40.21	44.82	3.46	280.32
TLG	55	294.16	208.18	15.15	824.14
NLR	55	4.15	2.96	0.79	17.11
PLR	55	211.10	114.21	49.79	651.25
CEA (ng/mL)	55	294.10	627.40	0.55	2890.90
CA 19-9 (U/mL)	55	3133.72	10478.41	0.80	596.53

MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, NLR: Neutrophils to Lymphocytes ratio, PLR: Platelets to Lymphocytes ratio, CEA: Carcinoembryonic antigen, CA19-9: Carbohydrate antigen 19-9.

CEA and CA19-9 were 0.676±0.074 (P=0.031) and 0.607±0.082 (P=0.189), respectively. As a result of ROC analyses the only significant P value (P=0.031) was obtained with CEA. However, CEA had not a very high AUC (0.676) value (Table 3).

In multivariate logistic regression analysis, the predictive power of age, SUVmax, SUVmean, MTV, TLG, NLR, PLR, CEA and CA 19-9 values in the prediction of KRAS gene mutation was evaluated. P-values and OR values are summarized in Table 4. No significant association was observed between these parameters and KRAS mutations.

Discussion

Although in recent years ^{18}F-FDG PET/CT has been used intensively, only a few studies have investigated the predictive capacity of semiquantitative parameters (SUVmax, MTV, TLG) based on PET/CT measurements for the prediction of KRAS mutation [11, 12]. We also investigated predictive capacities of hematological parameters as NLR and PLR, tumor markers CEA and CA 19-9 in the prediction of KRAS mutations and we observed a significant (though not very strong) association only between CEA and KRAS (P=0.03, AUC: 0.676).

***Table2**. Differences in values of SUVmax, MTV(cm^3), TLG, NLR, PLR, CEA(ng/mL) and CA19-9(U/mL) between the wild-type and mutant KRAS groups.*

Independent samples t-test

	KRAS	N	Mean	Std. dev.	P
SUVmax	Wild-type	35	16.57	9.22	0.933
	Mutant	20	16.78	7.26	
SUVmean	Wild-type	35	8.58	5.05	0.879
	Mutant	20	8.79	4.24	
TLG	Wild-type	35	275.22	214.59	0.377
	Mutant	20	327.31	197.35	
PLR	Wild-type	35	192.31	97.87	0.107
	Mutant	20	243.99	134.69	

Mann-Whitney U test

	KRAS	N	Median	IQR	P
MTV (cm^3)	Wild-type	35	26.75	21.82	0.263
	Mutant	20	29.48	45.91	
CEA (ng/ml)	Wild-type	35	6.96	248.17	0.031
	Mutant	20	55.52	317.58	
CA19-9 (U/ml)	Wild-type	35	28.20	289.60	0.189
	Mutant	20	132	1621.75	
NLR	Wild-type	35	3.17	1.79	0.056
	Mutant	20	4.08	4.50	

MTV:Metabolic tumor volume, TLG:Total lesion glycolysis, NLR:Neutrophils to Lymphocytes ratio, PLR:Platelets to Lymphocytes ratio, CEA:Carcinoembryonic antigen, CA19-9:Carbohydrate anti-gen 19-9.

***Table 3.** Shows ROC analysis, AUC (areaundercurve), standard error, confidence interval and Pvalues of SUVmax, SUVmean, MTV, TLG, NLR, PLR, CEA and CA19-9.*

	AUC	Standard error	%95 Confidence interval		P value
SUVmax	0.543	0.080	0.385	0.700	0.600
SUVmean	0.543	0.080	0.386	0.699	0.600
MTV (cm^3)	0.591	0.081	0.432	0.751	0.263
TLG	0.601	0.077	0.451	0.752	0.214
NLR	0.656	0.081	0.496	0.815	0.057
PLR	0.633	0.079	0.478	0.788	0.104
CEA (ng/mL)	0.676	0.074	0.531	0.820	0.031
CA 19-9 (U/mL)	0.607	0.082	0.447	0.767	0.189

MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, NLR: Neutrophils to Lymphocytes ratio, PLR: Platelets to Lymphocytesratio, CEA: Carcinoembryonic antigen, CA19-9: Carbohydrateantigen 19-9.

SW Chen et al. (2015) [12] investigated the association between SUVmax, MTV, TLG, tumor width (TW) which are ^{18}F-FDG PET/CT parameters and various genetic mutations (TP53, KRAS, APC) in a study they performed on 103 patients and observed that only TW could predict independently KRAS gene mutations. SUVmax, MTV and TLG did not independently predict KRAS mutation per se, as in our study.

The same authors [12] determined various thresholds when calculating MTV and TLG (SUVmax, 40%; SUVmax, 30%; SUVmax:2.5 etc.). They estimated threshold of TW value which they found to be significant for KRAS mutations as was SUVmax in 40 percent. We also determined a threshold (SUVmax, 40%) when calculating MTV and TLG. In fact, we planned to perform comparisons by determining threshold value as SUVmax: 2.5. However, when we considered as a threshold value 2.5, we experienced problems of the physiologic FDG uptake of the colon and vesical activity in autocontouring large tumors in the rectum. At the beginning, thresholds as SUVmax 40% and 2.5 were planned for statistical analyses, but due to the above mentioned problems, only SUVmax 40% threshold was finally determined in our study.

Table 4. *Multivariate logistic regression analysis for the prediction of KRAS gene mutations.*

	Univariate P value	Multivariate P value	OR	95% CI for OR	
				Lower	Upper
Age	0.078	0.458	0.988	0.958	1.019
SUVmax	0.077	0.365	0.850	0.598	1.208
SUVmean	0.336	0.636	0.992	0.961	1.025
MTV (cm^3)	0.088	0.501	1.258	0.645	2.456
TLG	0.247	0.451	1.003	0.996	1.009
NLR	0.688	0.149	1.384	0.890	2.152
PLR	0.297	0.403	0.996	0.988	1.055
CEA(ng/mL)	0.875	0.240	0.999	0.997	1.001
CA 19-9(U/mL)	0.196	0.355	1.000	1.000	1.000

MTV:Metabolictumorvolume,TLG:Totallesionglycolysis,NLR:NeutrophilstoLymphocytesratio,PLR:PlateletstoLymphocytesratio,CEA: Carcinoembryonicantigen,CA19-9:Carbohydrateantigen19-9.

Kawada et al. (2012) [11], performed a retrospective study on 51 colorectal cancer patients and investigated the correlation between SUVmax and tumor to liver ratio (TLR) derived from PET/CT imaging and KRAS/BRAF mutations and observed significantly higher SUVmax and TLR in colorectal cancer patients with KRAS/BRAF mutations than in those with KRAS wild-type mutation. (P=0.006 and P=0.001, respectively). They also estimated that when cut-off values of SUVmax of 13 and 14 were taken as a basic reference, SUVmax could predict KRAS/BRAF mutation with a 75% accuracy. However, in our study, SUVmax did not differ significantly in patients with mutant or wild-type KRAS gene.

Zy Chen et al. (2014) [20] performed a study on 342 colorectal cancer patients and detected KRAS mutation in 52.6% of them. However, they did not find a significant correlation between NLR and KRAS mutation (OR:0.98; 95% CI: 0.57-1.69; P=1.000). Similarly, in our study, a significant correlation did not exist between KRAS and NLR. Very few studies in the medical literature have disclosed NLR and KRAS mutation. The study performed by Chen et al. 2015 [20] is the only study we could find in the literature which investigated this correlation. From this perspective, our study contributes to the literature.

Some studies have demonstrated the prognostic value of NLR and PLR in colorectal cancers [18]. In some studies, the correlations between NLR, PLR and PET parameters have been investigated. In a study performed by Surucu et al. (2015) [21] in patients with esophageal cancers, the correlations between MTV and hematological parameters were investigated and a correlation between NLR and MTV was observed. (P= 0.013). Although not indicated in the "Results" section we also couldn't find significant correlations between PET parameters and NLR, PLR in colorectal cancer patients. We obtained P values between NLR-SUVmax, NLR-MTV and NLR-TLG which were 0.586, 0.443 and 0.824, respectively. The P values between PLR-SUVmax, PLR-MTV and PLR-TLG were 0.898, 0.400 and 0.107 respectively.

Tumor markers as CEA and CA 19-9 have been used in the monitorization, diagnosis, staging, evaluation and determination of recurrences [22]. However, in the literature, very few studies have evaluated the correlation between tumor markers and KRAS oncogene [23, 24]. Selcukbiricik et al. (2013) [23] investigated 215 patients with colorectal cancer and similarly to our study, observed a significant difference between CEA values in patients carrying mutant KRAS gene and wild-type gene (P=0.02).

Li et al. (2015) [24] investigated 945 patients and observed a significant association between KRAS mutations and CEA, CA 19-9 (P=0.0001). Still in our study when ROC curve for CEA was drawn based on the reference of KRAS mutations, we obtained a significant P value (P=0.03), but AUC (0.676) was not very high. Besides, in multivariate logistic regression analysis, P value (0.240) was 0.24.

Caglar et al. (2015) [25] investigated correlations between SUVmax, MTV, TLG, and serum CEA levels in 155 recurrent colorectal cancer patients and a correlation with moderate level of significance was observed (P<0.001; cor-relation coefficients: 0.45, 0.44 and 0.49, respectively). They also sought for a correlation between CA 19-9 and SUVmax, MTV,

TLG; a moderate level of significance was detected between CA 19-9 and TLG, MTV (P<0.001; correlation coeffi-cients: 0.49 and 0.48, respectively). We could not find signi-ficant correlations between PET parameters and CEA, CA 19-9.

In the present study, patients with colorectal cancer were retrospectively screened. Information about histopathological subtypes and pathological stages of colorectal cancers of some patients could not be obtained. Therefore, we did not divide and evaluate patients according to their histopathological subtypes and pathological stages. According to the literature, the most frequently observed subtype (90%) of colorectal carcinomas are adenocarcinomas. Other rare types are squamous cell, neuroendocrine, spindle cell, adenosquamous and undifferentiated carcinomas. Studies performed in histopathological subgroups may yield different outcomes from our results.

In conclusion, in our study, we have observed that in patients with colorectal cancers, we cannot predict KRAS gene mutations using ^{18}F-FDG PET/CT parameters (SUVmax, MTV, TLG), hematological parameters (NLR, PLR) or tumor marker CA 19-9. We detected a significant but not a very strong associ-ation between only CEA and KRAS mutations. Future, studies performed with greater number of patients categorized into subtypes will yield more significant results.

The authors of this study declare no conflict of interest

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