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Original Article

Association between 18F-fluorodeoxyglucose-PET/CT and histological grade of uterine endometrial carcinoma

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ABSTRACT

Objective: The incidence of endometrial adenocarcinoma of the uterine corpus has increased in Japan. This study aimed to clarify the relationships between this type of cancer and various data provided by 18F-fluorodeoxyglucose (FDG) accumulation in positron emission tomography/computed tomography (PET/CT).
Materials and methods: The study cohort thus comprised 27 patients with endometrial adenocarcinoma who had undergone PET/CT examinations from April 2008 to March 2015. All patients provided informed consent at our hospital. Data from 27 patients with endometrial adenocarcinoma (Grades 1–3) were retrospectively analyzed to determine the relationships between the maximum standardized uptake value (SUVmax), histological grading, tumor size, and rate of positivity for glucose transporter 1, hexokinase II, and glucose-6-phosphatase-α (G6Pase-α).
Results: SUVmax values differed significantly between patients with Grade 1 (G1) and Grade 2 (G2) or higher cancer (P = 0.031). For G1 cancer, a negative correlation was found between SUVmax and G6Pase-α (R = −0.475, P = 0.046). The regression coefficient for G6Pase-α was −0.125 (95% CI: −0.165 to −0.084) and the P-value 0.008; thus this difference was significant.
Conclusion: PET/CT is a useful test for discriminating between G1 and G2 or higher cancer in patients with endometrial adenocarcinoma of the uterine corpus. In addition, the negative correlation identified between SUVmax and G6Pase-α activity in patients with well-differentiated endometrial cancer may be a novel finding.
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Introduction

Positron emission tomography/computed tomography (PET/CT) is a very useful means of diagnosing malignant tumors [1]. The radiopharmaceutical 18F-fluorodeoxyglucose (FDG) aids assessment of PET/CT images by accumulating in cancer cells. The maximum standardized uptake value (SUVmax) is a quantitative measure of FDG accumulation.

The incidence of endometrial cancer has been increasing, even in young women, reportedly because of high-fat/high-protein diets, low birth rate, and older age at first birth [2]. In Japan, endometrial adenocarcinoma accounts for approximately 90% of endometrial cancers [3]. The degree of differentiation of these cancers is an important prognostic factor, greater differentiation being associated with a better prognosis [4].

Our attention was drawn to the possible significance of the maximum standardized uptake value (SUVmax) when we encountered several tumors with extremely low SUVmax, even when the surgical endometrial tumor samples were 13 mm or larger. All such patients were diagnosed with Grade 1 endometrial cancer on endometrial tumor samples and we observed that Grade 1 endometrial cancers detected by FDG-PET/CT at our hospital had a significantly lower SUVmax than Grade 2 or higher cancers.

In this study, we therefore aimed to identify the clinical factors associated with FDG accumulation in patients with endometrial cancer to ascertain whether these associations could facilitate early detection. We chose to investigate antibodies that are associated with FDG metabolism by metabolic trapping. Glucose metabolism is increased in cancer cells and these cells take up FDG, which has a similar molecular structure to glucose, via a glucose transporter. The incorporated FDG becomes phosphorylated by hexokinase and remains in the tumor cells. However, phosphorylated fluorodeoxyglucose-6 is dephosphorylated in glucose-6-phosphatase (G6Pase-α) -rich tumors and subsequently eliminated from them. Given this glucose metabolism-related mechanism of accumulation of FDG, we examined the relationship between the SUVmax and various tissue characteristics by immunohistochemical staining of samples of Grade 1, 2, and 3 tumors.

Methods

Patients

This retrospective study evaluated 33 consecutive patients who received PET/CT within 4 weeks prior to surgery, from April 2008 to March 2015. Exclusion criteria were as follows: presence of comorbidity that affects the SUVmax such as diabetes mellitus, which is characterized by high blood sugar concentrations [5]; presence of uterine fibroids near the endometrium; and menstruating women in whom PET/CT is contraindicated to avoid physiologic FDG uptake. Six patients were excluded on the basis of these criteria (Fig. 1).

The study cohort thus comprised 27 patients with endometrial adenocarcinoma who had undergone PET/CT examinations from April 2008 to March 2015. All patients provided informed consent at the Obstetrics and Gynecology Department of Kanazawa Medical University Hospital (Kanazawa, Japan).

All included patients were histologically confirmed as having uterine corpus endometrial adenocarcinoma and were aged 30–84 years (mean $\pm$ standard deviation, 60.7 $\pm$ 12.15 years; median age, 62.0 years). Definitive diagnoses were made by histopathologic examination of surgical specimens.

PET/CT procedure

All PET/CTs were performed with a Biograph Sensation 16 scanner (Siemens, Bayern, Germany). After at least 6 h of fasting, the patients were given 185-MBq FDG intravenously. Sixty minutes later, a low-dose non-contrast CT scan was performed for attenuation correction and to collect anatomical information. The PET images had a matrix size of 256 $\times$ 256, which corresponds to a pixel size of 2.6 $\times$ 2.6 mm^2. PET data were reconstructed with an image resolution of approximately 6.5 mm full width at half maximum (FWHM). SUVmax was measured in regions of interest in the primary tumor. As to the precision of PET/CT, FDG emits positrons from fluorine 18 (F-18) and the radiation source size may be
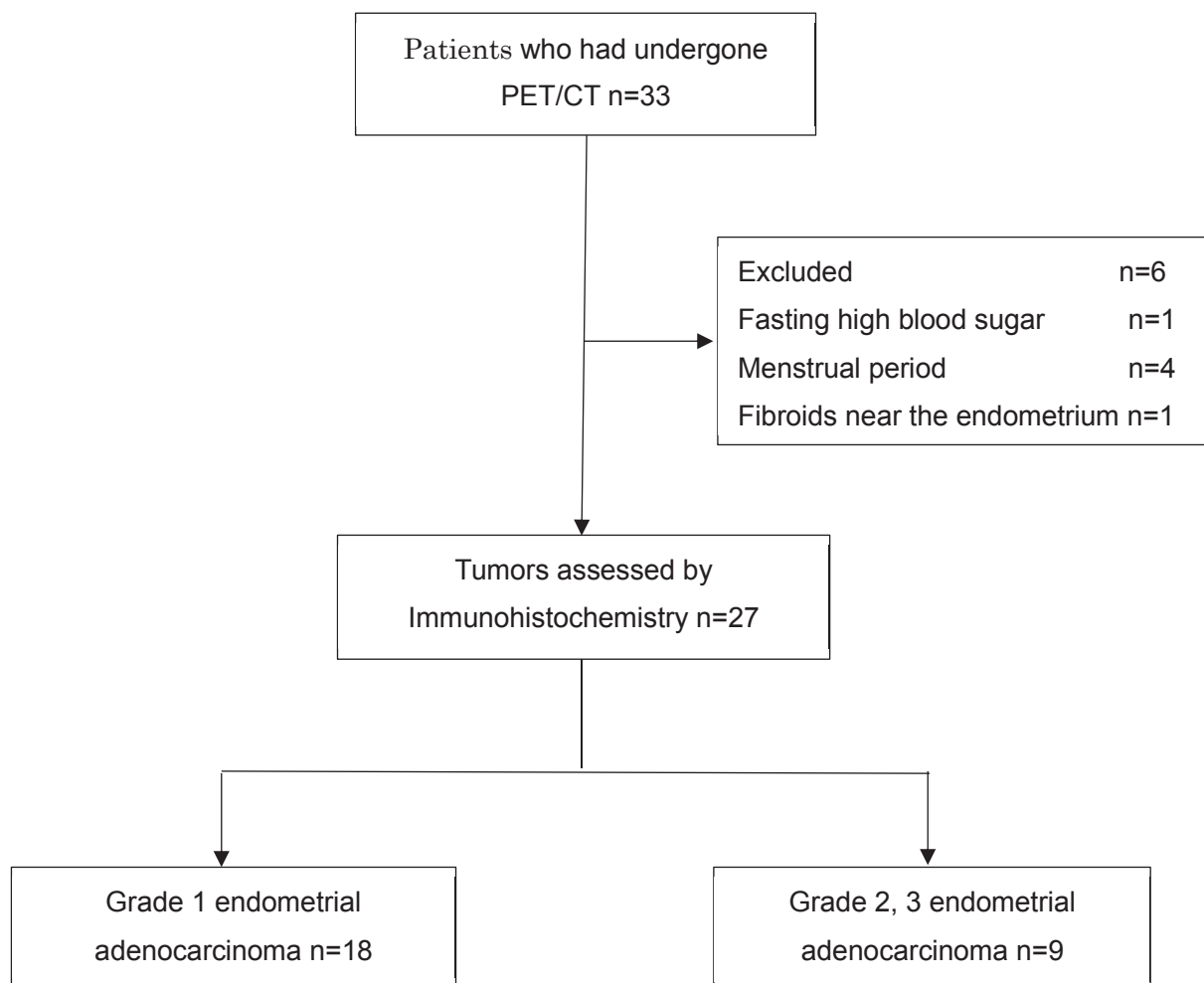

Fig. 1. Flowchart showing inclusion and exclusion criteria for patients with endometrial cancer.

affected by a partial volume effect until space resolution (i.e., FWHM) is duplicated [6]. The space resolution of PET/CT in our hospital is 6.3 mm and 7.1 mm when measured at 1 cm and 10 cm, respectively, from the tumor center. Because 13 mm or larger tumors are not affected by the partial volume effect, tumor sample size does not affect FDG accumulation in patients with endometrial adenocarcinoma and extremely low SUVmax.

The PET images were reconstructed with an ordered-subset expectation maximization iterative reconstruction algorithm (eight subsets, two iterations).

The SUVmax of each tissue sample was calculated by using the SUVmax in tissues in which FDG accumulation had been detected according to the following formula:

Radiation dose of the tissue (Bq/g)/[dose (Bq)/weight (kg)].

A PET/CT image from a typical endometrial cancer is shown in Fig. 2.

Measurement of tumor size

The size of each uterine corpus cancer lesion was determined by bidimensional measurements (i.e., the product of the longest diameter and the longest perpendicular diameter of each tumor).

Immunohistochemistry for SUV-related markers

Three micrometer sections from 10% buffered formalin-fixed paraffin-embedded blocks containing representative parts of each lesion were stained with hematoxylin-eosin staining and immunohistochemical stains. The endometrial adenocarcinomas were graded histologically according to the differentiation and architecture of their glandular cell components as follows: Grade 1 (G1), solid tissue comprises 5% or less of the adenocarcinomatous components; Grade 2 (G2), solid tissue comprises 6%–49% of the adenocarcinomatous components; and Grade 3 (G3), solid tissue comprises 50% or more of the adenocarcinomatous components. The degree of differentiation is increased by 1 if significant cellular atypia is identified (criteria of the Japan Society of Obstetrics and Gynecology). According to these criteria, 18 patients were classified as having G1 cancer, eight patients G2 cancer, and one patient G3 cancer.

To elucidate glucose metabolism [7], the presence of glucose transporter 1 (Glut 1) [8], hexokinase II (HK-II) [9], and glucose-6-phosphatase-α (G6pase-α) [10,11] in tumor tissue was assessed by immunohistochemical analysis as follows.

After deparaffinization of the formalin-fixed paraffin-embedded segments, the samples were incubated with Glut 1 in an ethylenediaminetetraacetic acid-based cell conditioning solution (pH 8.5, Ventana Medical Systems, Tucson, AZ, USA) at 100 °C for 30 min. The samples were then exposed to HK-II in Target Retrieval Solution (S2031; Dako, Glostrup, Denmark) in a water bath at 95 °C for 40 min, cooled at room temperature for 20 min, washed with water, and then immersed in distilled water. The samples were then treated with G6Pase-α in KN9 antigen activating solution (code number, KN-09001; Pathology Institute, Toyama, Japan) in a water bath at 95 °C for 40 min, washed with water after cooling at room temperature for 20 min, and immersed in distilled water. The assay for endogenous peroxidase was performed using 3% hydrogen peroxide solution at room temperature for 10 min. After washing with distilled water several times, the segments were equilibrated in KN buffer (KN-09002; Pathology Institute) at room temperature for 5 min. The primary antibodies (at room temperature) for Glut-1: anti-glucose transporter glut 1 antibody ab15309 (Abcam, Cambridge, UK); HK-II: anti-hexokinase II rabbit monoclonal antibody colon C64G5 (Cell Signaling Technology, Danvers, MA, USA); and G6Pase-α: anti-G6Pase rabbit polyclonal antibody (H-60): sc-25840 (Santa Cruz Biotechnology, Paso Robles, CA, USA) were used at a dilution of 1:400 and reaction time of 10 min, a dilution of 1:100 and reaction time of 30 min, and a dilution of 1:200 and reaction time of overnight, respectively. After washing with KN buffer several times, the secondary antibodies (at room temperature for 30 min) for Glut 1, HK-II, and G6Pase-α were detected with I-View Biotin Ig + SA-horse radish peroxidase (HRP) 3,3′-diaminobenzidine (DAB)+H_2O_2, Copper (Ventana Medical Systems) and Envision + Dual Link HRP-labeled/polymer reagent (K4061; Dako), respectively. After washing with KN buffer several times, the 3,3′-diaminobenzidine color was developed at room temperature for 10 min, followed by washing with distilled water. Hematoxylin staining was performed at room temperature for 3 min, after successive washing with water, dehydration, penetration, and inclusion [12].

Evaluation of staining results

The rate of immunohistochemistry positivity of neoplastic cells in segments of the lesions was measured by a pathologist twice using 5% increments to obtain average values.

Representative immunohistochemical staining (100% positive, 45% positive, and 10% positive) for G6Pase-α is shown in Fig. 3.
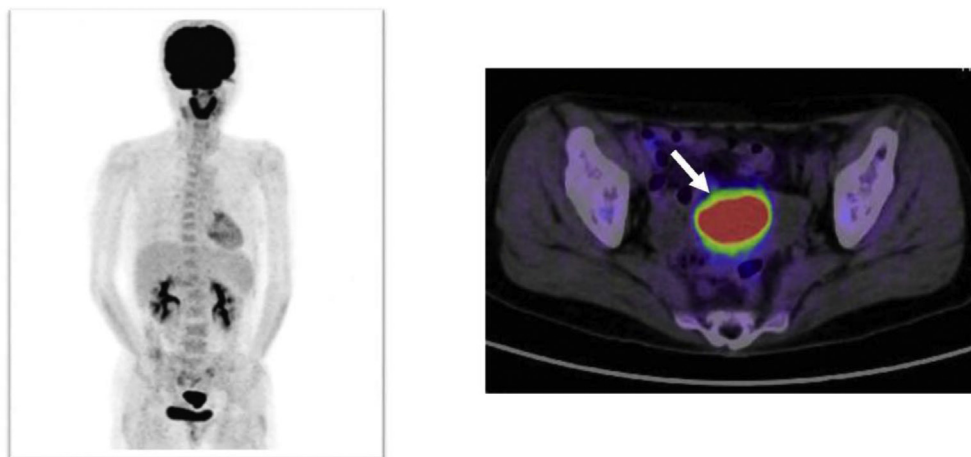
Fig. 2. PET/CT image of a typical endometrial cancer in a 50year-old woman with endometrial cancer Stage1b (Grade 1). The FDG PET image shows strong focal FDG accumulation (SUV = 19.88) in the tumor (arrow).
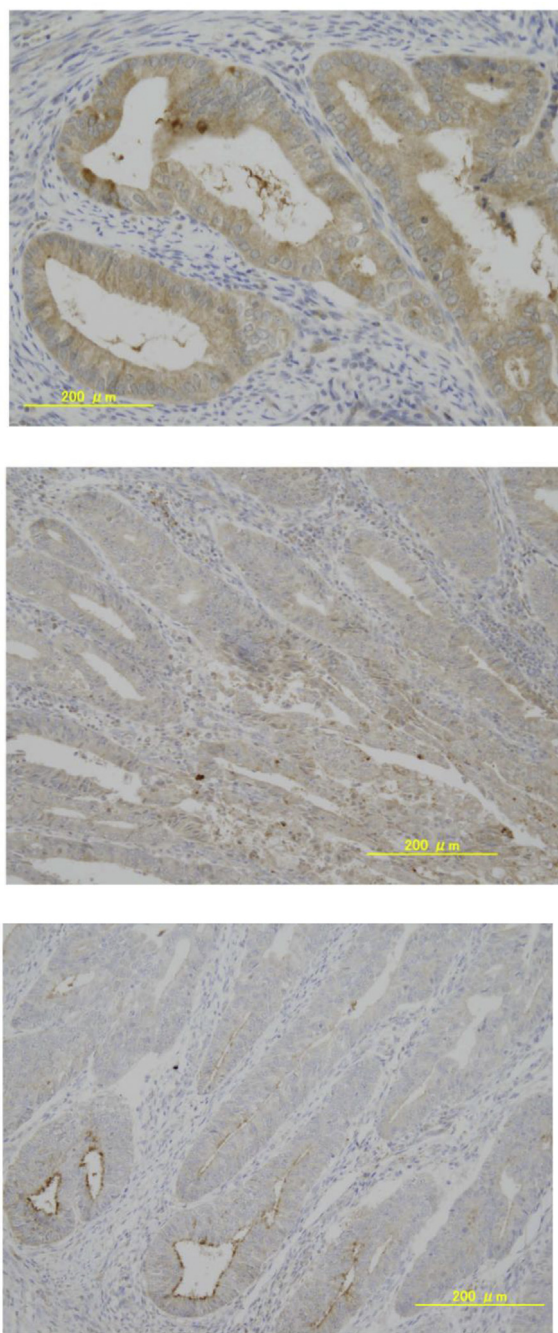
Fig. 3. Immunohistochemistry staining for G6Pase-α in an endometrial cancer. Case 12 (G1): 100% positive. Case 9 (G1): 45% positive. Case 8 (G1): 10% positive.

Statistical analysis

The relationship between the SUV of G1 and G2 or higher endometrial cancers, tumor size, and immunohistochemical findings (i.e., Glut 1, HK-II, and G6Pase-α) was examined by determining the correlation coefficient. R version 3.2.4 (R Core Team, 2016) was used for all statistical analyses. The level of statistical significance was set at $P \leq 0.05$. The Mann–Whitney U test and receiver operating characteristic (ROC) curves were used to assess differences between two independent groups. Spearman's rank correlation coefficient and multiple regression analysis were used for correlation analysis. Findings are presented as mean ± standard deviation unless otherwise stated.

Results

Table 1 shows the overall results for all study patients.

Mann–Whitney U test and ROC curve

The SUVmax of the 18 patients with G1 cancer was 10.50 ± 6.14 and of the nine with G2 and G3 cancers 17.36 ± 6.07; this difference is significant ($P = 0.031$). The AUC of the ROC curve was 0.759 with a 95% confidence interval (CI) of 0.562–0.957 and $P < 0.001$. Using a cut-off value of 16.07 for SUVmax, the sensitivity and specificity were 0.778 and 0.833, respectively (Fig. 4).

Spearman's rank correlation coefficient

In the whole cohort, there was a significant correlation between SUVmax and tumor size (Spearman's rank test, $R = 0.427$ and $P = 0.026$). However, no such correlation was identified between SUVmax and values for the enzymes associated with glucose metabolism (i.e., Glut 1, HK-II, and G6Pase-α).

For the 18 patients with G1 tumors, there was a significant correlation between SUVmax and tumor size ($R = 0.489, P = 0.039$) and between SUVmax and G6Pase-α values ($R = -0.475, P = 0.046$) (Fig. 5). By contrast, SUVmax did not correlate with Glut 1 or HK-II values.

For the nine subjects with G2 or G3 cancer, no values were significantly correlated with SUVmax. Specifically, the findings were as follows: SUVmax and tumor size ($R = -0.117, P = 0.765$), SUVmax and Glut 1 ($R = -0.100, P = 0.797$), SUVmax and HK-II ($R = 0.341, P = 0.370$), and SUVmax and G6Pase-α ($R = -0.034, P = 0.931$).

Univariate regression analysis

G6Pase-α
The regression coefficient was -0.106 (95% CI: -0.201 to -0.012) and $P = 0.030$, indicating a significant difference.

No other variables showed a significant difference.

Multiple regression analysis (linear regression model)

This analysis started with a full model incorporating nine explanatory variables. An optimum model was searched for based on the Akaike's Information Criterion (AIC) using the backward elimination method. This process resulted in selection of a model with the following six explanatory variables: diagnosis, pathological stage, blood glucose (BG) concentration, tumor size, HK-II, and G6Pase-α. The results of analysis by each of these variables are discussed below.

Diagnosis

Of the seven levels of diagnosis, only Stage Ib was significantly associated with SUVmax. The regression coefficient for Stage Ib was 6.794 (95% CI 3.855–9.733) and $P = 0.037$, indicating that the association was 6.794 times stronger for Stage Ib than for Stage Ia.

HK-II
The regression coefficient was 0.125 (95% CI: 0.067–0.183) and $P = 0.048$, indicating a significant correlation.

G6Pase-α
The regression coefficient was -0.125 (95% CI: -0.165 to -0.084) and $P = 0.008$, indicating a significant correlation.

Table 1
Details of data for all participants.

Case	Age (year)	Diagnosis	Pathological stage	BMI (kg/m²)	BG (mg/dl)	Tumor size (cm²)	GLUT1 (%)	HK-II (%)	G6Pase-α (%)	SUV
1	48	stageIa	grade1	26.6	89	2.5	25	95	10	20.36
2	68	stageIa	grade1	25.2	86	1.9	35	20	60	2.40
3	30	stageIa	grade1	20.3	85	4.5	75	90	90	1.95
4	58	stageIa	grade1	26.5	94	26.0	50	90	95	10.42
5	69	stageIa	grade1	26.4	84	5.3	65	95	80	5.42
6	54	stageIa	grade1	19.5	122	1.0	45	95	75	4.69
7	63	stageIa	grade1	21.1	129	10.5	20	65	45	9.61
8	45	stageIa	grade1	32.5	84	3.3	25	90	10	4.74
9	74	stageIa	grade1	31.1	110	5.0	55	95	45	11.67
10	44	stageIb	grade1	26.3	96	9.0	75	85	45	22.18
11	62	atageIb	grade1	23.5	109	13.4	70	50	20	13.92
12	62	stageIb	grade1	17.5	86	9.0	20	80	100	3.17
13	50	stageIb	grade1	23.6	89	12.2	35	85	45	20.44
14	75	stageIc	grage1	18.0	69	15.0	10	60	35	12.16
15	84	stageIc	grage1	26.6	108	24.3	40	25	35	11.77
16	64	stageIc	grade1	27.7	93	17.1	25	25	25	12.92
17	51	stageIIa	grade1	19.4	119	7.5	40	70	15	10.89
18	61	stageIIb	grade1	22.1	92	7.7	15	60	10	10.28
1	62	stageIb	grade2	21.4	85	1.3	95	50	25	10.85
2	63	stageIb	grade2	23.1	99	8.3	35	45	45	18.72
3	69	stageIb	grade2	24.4	101	15.0	70	85	40	30.39
4	59	stageIIb	grade2	23.5	123	15.8	15	45	90	8.94
5	53	stageIIb	grade2	17.5	74	41.3	85	70	40	16.07
6	72	stageIIIa	grade2	25.3	125	78.4	45	60	20	17.39
7	55	stageIIIc	grade2	22.0	99	11.4	60	50	35	16.11
8	60	stageIIIc	grade2	19.3	101	10.2	40	50	40	19.88
1	84	stageIIa	grade3	18.2	105	29.6	60	90	55	17.86

Body mass index; BMI, Blood glucose levels prior to PET/CT; BG.
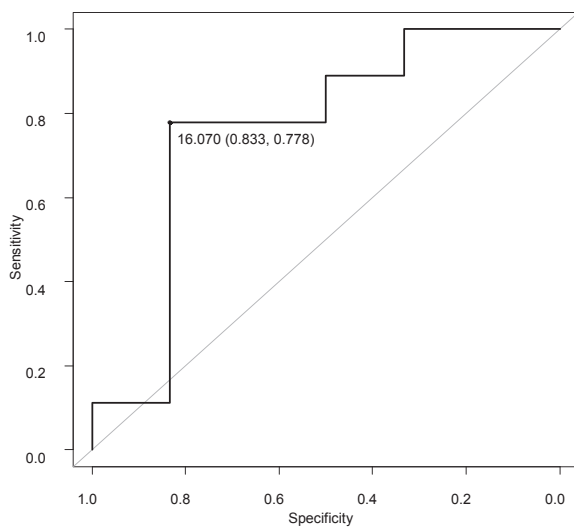
Fig. 4. ROC curve comparison between G1 and G2 or higher of endometrial cancer. SUVmax of the 18 patients with G1 tumors was 10.50 ± 6.14 and of the nine with G2 or higher 17.36 ± 6.07; this difference is significant (P = 0.031).

Fig. 5. Correlation of SUV and G6Pase-α in patients with G1 endometrial cancer. SUVmax and presence of G6Pase-α are significantly correlated (Spearman's Rank test, R = −0.475, P = 0.046).
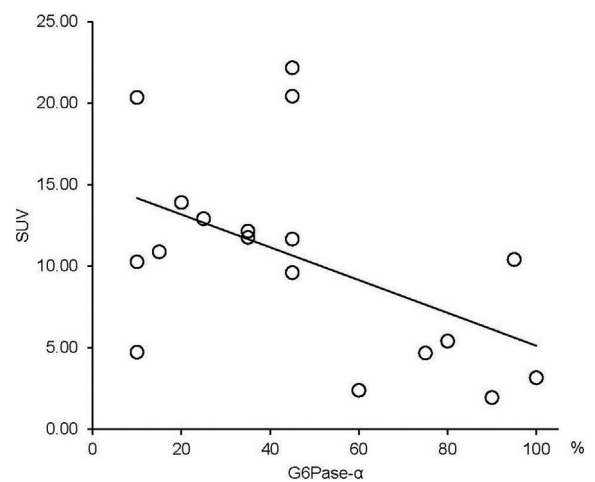
Pathological stage, BG, and tumor size

The P-values for pathological stage, BG, and tumor size were all >0.05; however, the following tendencies were noted:

Pathological stage

The regression coefficient for Grade 2 was 5.017 (95% CI: 1.703−8.330) and P = 0.152 and for Grade 3 was 5.042 (95% CI: −2.660 to 12.744) and P = 0.523.

BG

The regression coefficient was 0.095 (95% CI: 0.023−0.167) and P = 0.209.

Tumor size

The regression coefficient was 0.260 (95% CI: 0.114−0.406) and P = 0.097.

The contribution of this model to the variable (explanatory power) was 0.724 (>0.7), suggesting that a model with good explanatory power had been constructed.

Discussion

The study aim to clarify the relationship between histological grade of endometrial adenocarcinoma and various data associated with FDG accumulation in PET/CT imaging in 27 patients with endometrial cancer (G1−3). We analyzed the relationships between SUVmax and histological grading, tumor size and selected immunohistochemical findings.

According to the Mann–Whitney U test, SUVmax values differed significantly between patients with G1 and G2 or higher cancer.

The Spearman rank-order correlation coefficient indicated a significant positive correlation between tumor size and SUVmax. However, according to multiple regression analysis, tumor size is not associated with SUVmax, necessitating further exploration of the relationship between SUVmax and tumor size. We found a negative correlation between SUVmax and presence of G6Pase-α in G1 cancers. Subsequent multiple regression analysis indicated that presence of G6Pase-α in these cells may be linked with SUVmax, suggesting that G6Pase-α regulates FDG accumulation in patients with well-differentiated (i.e., G1) endometrial cancers.

Nakamura et al. [13] have reported finding a significant relationship between SUVmax and FIGO grade in endometrial cancers. Additionally, PET/CT imaging can reportedly be used to identify candidates for surgical staging with high sensitivity [14].

Some investigators believe that FDG does not readily accumulate in well-differentiated hepatocellular cancer because they retain the function of normal hepatic cells and have high G6Pase activity [15–17]. FDG is dephosphorylated and excreted from the cells of highly differentiated cancers [18]. Glucose-6-phosphatase is primarily distributed in the liver, kidney, and small intestine. However, Ockerman [19] has reported that G6Pase is also present in uterine mucous membranes. Therefore, FDG accumulation in endometrial cancer may be determined by a similar cellular metabolism, resulting in strong expression of G6Pase in well-differentiated tumors.

One limitation of our retrospective single-center study is the relatively small patient cohort. Another major limitation is the heterogeneity of the participants.

In conclusion, we identified a significant difference in SUVmax between G1 and G2 or higher endometrial adenocarcinomas. This finding may be useful for predicting the histological differentiation. We believe that our identification of a negative correlation between SUVmax and G6Pase-α activity in patients with well-differentiated endometrial cancer is a novel finding.

Conflicts of interest statement

The authors declare no conflict of interest associated with this manuscript.

Acknowledgment

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