Clinical significance of ^{18}F-fluorodeoxyglucose position emission tomography in superficial esophageal squamous cell carcinoma

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ABSTRACT

Background: The aim of this study was to assess the clinical usefulness and significance of 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) in superficial esophageal squamous cell carcinoma (ESCC).

Patients and Methods: We examined FDG-PET for 80 consecutive patients with superficial ESCC without neoadjuvant treatment. Fifty seven patients underwent radical esophagectomy and 23patients received endoscopic resection. Then FDG-uptake index was evaluated with clinicopathological findings and Glucose transporter 1 (Glut-1) expression in primary tumors was examined immunohistochemically.

Result: The FDG-uptake in primary tumors correlated with histology, depth of tumor invasion, lymph node metastasis, lymphatic invasion, vascular invasion and Glut-1 expression. All patients with more than 4.4 maximum standardized uptake value (SUV max) had deeper invasion of submucosa. Among 16 patients with lymph node metastasis, only two patients were detected lymph node metastasis. FDG-uptake, depth of tumor invasion, lymph node metastasis, and histology were found to be prognostic factors and histology was an independent prognostic factor. In FDG uptake positive patients, depth of tumor invasion and histology were prognostic factors.

Conclusions: FDG-PET is useful for diagnosing tumors with deeper invasion of submucosa, and helpful for the decision making of the endoscopic treatments for superficial ESCC. Moreover, patients with FDG uptake positivity, deeper invasion of submucosa, and poorly differentiated tumor and had poor prognosis should treat with multimodal treatment.

Key words :

FDG-PET, superficial esophageal squamous cell carcinoma, lymph node metastasis, Glut-1

Running title: PET in superficial ESCC

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INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive cancers of gastrointestinal tracts. It frequently metastasizes to the lymph nodes and other organs resulting in a poor prognosis[1,2]. For the decision of treatment strategy of ESCC, it is important to evaluate depth of tumor invasion, lymph node or hematogenous metastasis by preoperative precise diagnosis. When tumors invade into mucosal layer or shallow submucosal layer without lymph node metastasis, endoscopic mucosal resection (EMR) [3] or an endoscopic submucosal dissection(ESD) [4] are applicable. Although recent advanced endoscopic examinations enables us to diagnose superficial ESCC[5], it is still hard to correctly diagnose mucosal or submucosal invasion.

18F-fluorodeoxyglucose positron emission tomography(FDG-PET) can detect enhanced glycolysis of tumor cells and has been proven valuable in diagnosing, staging, detecting recurrences, and assessing response to therapy in a multitude of malignant disorders[6]. Increased glucose uptake is found in malignant tumors because of increased levels of both glucose transporters (Gluts), and cellular concentration of FDG in a tumor represents the glycolytic activity of viable tumor cells[7]. Several reports showed to be closely related between tumor growth and FDG uptake on PET [8]. FDG-PET is useful for staging of advanced esophageal cancers before treatment or evaluating the response to neoadjuvant chemotherapy and chemoradiation therapy[9,10]. However, there are a few studies on the clinical value of FDG-PET in superficial ESCC[11] and is no report about the correlation between the Glut1 expression and FDG-PET uptake without chemotherapy or chemoradiation therapy.

In present study, we determined several clinical new findings regarding FDG-PET in superficial ESCC. Moreover, our study show that the new clinical investigation using FDG-PET including about lymph node metastasis and the correlation between Glut-1 expression and FDG uptake in a large number of superficial ESCC.

MATHERIALS AND METHODS

Patients

The subjects were 80 patients with ESCC(73 males and 7 females) who underwent esophagectomy or endoscopic mucosal resection(EMR) or endoscopic submucosal dissection(ESD) between 2006 and 2011 at Kagoshima University Hospital, Kagoshima, Japan. The patients ranged in age from 44 to 86 years (mean, 66.5 years). None of these patients underwent chemotherapy or radiotherapy before treatment, and none of them had synchronous or metachronous multiple cancer in other organs. All patients were checked out the abnormal glucose tolerance by blood examination and those with diabetes mellitus or abnormal glucose tolerance were not included in this study. 57 patients underwent radical esophagectomy, 17 patients received endoscopic submucosal resection(ESD), and the remaining 6 underwent endoscopic mucosal resection(EMR). Specimens of cancer tissues and noncancerous adjustment tissue were collected from the patients after informed consent had been obtained in accordance with the institutional guidelines of our hospital. Tumor staging was classified according to the tumor node metastasis classification of the International Union Against Cancer[12].

The depth of cancer invasion was histologically subdivided as follows; the depth of cancer invasion was subdivided histologically into three categories; M1- carcinoma *in situ* (intra-epithelial carcinoma); M2- cancer invasion confined to the lamina propria; M3- cancer reaching to or infiltrating into the muscularis mucosae; SM1 – invasion limited to the upper one-third of the submucosa; SM2 – invasion to between one-third and two-thirds of the submucosal depth; SM3 – invasion into the deepest third of the submucosa (Fig. 1).

The PET/CT protocols

PET was performed using a PET/CT system (Discovery STE ; GE Medical Systems, Milwaukee, WI, USA). All patients were instructed to fast for at least 5 hours before PET imaging. Image acquisition for the whole-body scans started 1h after the intravenous administration of FDG (3.7 MBq/kg body weight). Initially, CT scans from the brain to the pelvis were performed immediately prior to the PET scan with a multi-detector spiral CT scanner (3.75 mm slice thickness, a pitch of 1.75, 120 keV and 30–200mA depending on the patient's total body mass). Whole-body PET scans were performed, covering an area identical to that covered by CT. Acquisition time was 2.5min per bed position with 8 bed positions. The attenuation-corrected FDG PET images using the CT data were reconstructed with an ordered-subset expectation maximization algorithm (28 subsets, 2 iterations).

The attenuation-corrected PET,CT and fused PET/CT images were available for review in axial, coronal and sagittal planes, as was a cine display of maximum intensity projection(MIP) of the PET data, using the workstation (Advantage Windows Workstation ; GE Healthcare, Milwaukee, WI, USA).

Image analysis

Three radiologists reviewed, interpreted and analyzed all of the PET/CT images via consensus. The PET/CT images were analyzed visually and semi-quantitatively. FDG uptake was classified into heterogeneous and homogeneous patterns. The spotted or mottled FDG uptake was defined as the heterogeneous pattern, and the homogeneous FDG uptake in the whole tumor as the homogeneous pattern. Semiquantitative analysis of a lesion was performed by measuring the maximum standardized uptake value (SUVmax). The SUVmax was calculated using the commercially available soft ware provided by the manufacturer. The SUVmax for each ESC was automatically recorded by drawing a rectangular three-dimensional region of interest over the largest area of abnormal FDG uptake in the ESC lesion to cover the entire tumor volume. The FDG uptake of tumor is visible when the SUVmax is above approximately 2.5. Thus, cases with SUVmax in primary tumor > 2.5 were judged as PET positive.

Immunohistochemical Staining and Evaluation

The specimens were cut into 3-Am-thick sections, which were mounted on glass slides. Immunohistochemical staining was done using the avidin-biotin-peroxidase complex method (Vectastatin Elite ABC Kit;Vector, Burlingame,CA), following the manufacturer's instructions. Briefly, the immunostaining was performed manually at room temperature. The sections were deparaffinized in xylene and dehydrated in ethanol, endogenous peroxidase activity was blocked by incubating sections for 10 minutes in 3% hydrogen peroxide in methanol. Then, the sections were heated in a citrate buffer (0.01 mol/L, pH 6.5) at 121C for 15 minutes (autocrave) to reveal the antigen. After cooling, the sections were preincubated in 1% BSA for 20 minutes. Next, sections were incubated with anti-Glut-1 rabbit monoclonal antibody (1:100, Glut-1, Immuno-Biological Laboratories Co., Ltd, Fujioka.Japan) for 60min. After rinsing with

PBS for 15mim, the sections were incubated with secondary antibody for 20 min and washed again. After washing with PBS for 10 min, sections were incubated with avidin-biotin complex for 30 min and washed again, and reactions were visualized using diaminobenzidine tetrahydrochloride for 2 min. All samples are were lightly counterstained with hematoxylin for 1 min. No antigen retrieval was performed. Positive controls and negative controls were used for each section.

Evaluation of immunohistochemistry was independently done by two investigators (K.Y. and H.O.). Glut-1 positive expression was defined as detectable immunoreaction in cell membrane regions of >10% of the cancer cells. To evaluate expression of Glut-1, 10 fields (within the tumor and at the invasive front) were selected and expression in 1,000 tumor cells (100 cells/field) was evaluated using high-power (×200) microscopy.

Statistical Analysis

The statistical analysis of group differences was performed using the χ^2 test, the Student's t- test or Mann Whitney-U analysis. Overall survival curves were plotted according to the Kaplan-Meier method, with the Wilcoxon test applied for comparisons. $p < 0.05$ was considered statistically significant. Variables with a value of $p < 0.05$ by univariate analysis were used in subsequent multivariate analyses based on Cox's proportional hazards model. All statistical analyses were performed using JMP™ for Windows (Version 5.0.1, SAS Institute Inc, Cary, NC, USA).

RESULT

Relationship between FDG uptake in primary tumor and Clinicopathologic Variables

Among 80 patients with superficial ESCC, 35 patients had positive FDG uptake.

FDG uptake was significantly associated with the following clinicopathologic parameters: histopathological type, depth of tumor invasion, lymph node metastasis, lymphatic invasion, venous invasion and Glut-1 expression (Table 1A).

Relationship between SUV max value and clinicopathologic variables in FDG uptake positive patients

In the FDG uptake positive 35 patients, SUV max value was distributed from 2.8 to 14.5 and mean value was 5.3. Depth of tumor invasion was significantly associated with SUV max value (P=0.035). SM2-3 tumors had higher SUV max value than M1-SM1 tumors (Table1B). Mean SUV max value in M1-SM1 tumors was 3.6, however that in SM2-3 tumors was 5.6. All 19 patients with FDG uptake positive tumors more than 4.4 SUV max value had SM2-3 tumors (Fig. 1).

Relationship between FDG uptake and metastatic lymph node status

Sixteen out of 80 patients (20.0%) were diagnosed as having lymph node metastasis. Among them, in only two patients with SM3, lymph node metastasis was detected by FDG-PET (Table 3).

Expression of Glut-1 in superficial ESCC

Glut-1 expression was found in both cytoplasm and cell membrane of ESCC cells in 91.3% (73 of 80) of all patients (Fig. 3). The incidence of Glut-1 positive expression in cell membrane was 30% (24 of 80). FDG uptake was significantly associated with Gult-1 expression in cell membrane, although there was no correlation between the cytoplasmic expressions of Glut-1 and FDG uptake (Table 1A).

Survival analyses

In univariate analyses, FDG-uptake, depth of tumor invasion, lymph node metastasis, and histology were found to be prognostic factors (Table3). Multivariate regression analysis indicated that histology was an independent prognostic factor (Table 3). In FDG uptake positive patients, depth of tumor invasion and histology were prognostic factors (Fig. 2).

DISCUSSION

There have been reported that FDG-PET is a useful for diagnosis of staging or predicting a prognosis of patients with ESCC, although a few previous studies reported the clinical significance in superficial ESCC[11].

In a comparison with clinicopathologic variables, FDG uptake correlated with the histology, depth of tumor invasion, lymph node metastasis, lymphatic invasion and vascular invasion. SUV max value was significantly associated with SM2-3 invasion significantly and tumor with more than 4.4 SUV max value can be diagnosed as SM2-3 tumor. Miyata et al. [11] reported that FDG uptake correlated well with depth of tumor invasion and lymph node metastasis in 41 surficial ESCC, which was concordant with our present study. From these data, we should not perform endoscopic treatment for FDG positive tumor, especially in tumors with more than 4.4 SUV max value tumor. Clinically, it is really difficult and important to diagnose accurately depth of tumor invasion before treatment. For accurate diagnosis, we usually perform endoscopy (including magnifying endoscopy with narrow-band imaging) and endoscopic ultrasonography (EUS) for all superficial ESCC cases before treatment.

In the diagnosis of lymph node metastasis of esophageal cancer, many studies have demonstrated that in the diagnostic sensitivity, specificity, and accuracy rates in

esophageal cancer, PET cannot distinguish adjacent lymph node metastases of primary tumor because of poor spatial resolution and partial volume effect[13]. Moreover, In primary papillary thyroid cancer, Tumor size can influence FDG-PET findings and independent variables for false negative findings[14]. In our study on superficial ESCC, we could not prove the usefulness of FDG-PET for detecting lymph node metastasis. Our result was concordant with those previous studies. Regarding as false negative case, the size of the lymph nodes that have metastasis were less than 10mm. Moreover, the sizes of two FDG-uptake positive lymph nodes were 23 x 18mm and 12 x 10mm respectively. We suppose that the size of metastatic lymph node, namely tumor volume in the metastatic nodes, is important to diagnose as metastasis by FDG-PET. On the other hand, Kato et al [15] showed that the number of PET-positive lymph nodes was found to be an independent prognostic factor. From these data, FDG-PET was not effective for detecting lymph node metastasis, however it may be effective to predict the prognosis in superficial ESCC. We previously reported that ultrasonography (US) and EUS are useful modalities for diagnosis of the and lymph node metastasis as well as depth of tumor invasion before treatment in superficial ESCC [16,17]. Those devices are more sensitive than CT or MRI, because they are able to detect small size lymph nodes and its internal structure simultaneously.

Cancer cell have higher rates of glucose metabolism than normal cells[18], and malignant tissues typically demonstrated higher FDG-uptake than benign or normal tissues[19]. Glut-1 is the human erythrocyte glucose transporter and is also localized to the perineurium, brain, placenta, renal tubes, and germinal center cells in reactive lymphoid tissue[20]. Several immunohistochemical studies have demonstrated overexpression of

Glut-1 in human malignancy and a correlation between Glut-1 expression and neoplastic progression in human malignancy of colon[21], breast[22],liver [23], and esophagus[24].In esophageal cancer, Toma et al. reported that Glut-1 expression was a possible independent predictive value of prognosis[25]. In addition to those reports, our present study showed that the Glut-1 expression in the tumor cell membrane correlated with the FDG uptake, although there was no correlation with SUV max value or prognosis. Roadl et al. [26] reported that the total lesion glycolysis calculated by multiplying the tumor volume by the mean SUV of the volume was a better predictor of histopathologic response and survival than the decrease of the SUV value. Those results were almost concordant with our present study. Further study will be required to better understand the clinical and prognostic significance of Glut-1 expression and FDG-PET uptake in ESCC.

In survival analyses FDG uptake, depth of tumor invasion and histology were prognostic factors, and histology was an independent prognostic factor (p=0.002). Among FDG uptake positive patients alone, patients with SM2-3 tumor and poorly differentiated tumors had poor prognosis. Therefore we should treat these patients with multimodal treatment such as neoadjuvant chemotherapy and/or radiotherapy from now on.

In conclusion, FDG-PET is useful for diagnosing SM2-3 tumors, and helpful for the decision making of the endoscopic treatments for superficial ESCC. Moreover, since patients with positive FDG uptake positive, SM2-3 and poorly differentiated tumors and had poor prognosis, they should be treated with multimodal treatment.

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Figure legends

Figure 1

Evaluation of SUV-max value in FDG uptake positive patients

Mean SUV max value in m1-sm1 tumors (n=5) was 3.62, however that in SM2-3 tumors (n=30) was 5.64. All patients with FDG uptake positive tumors more than 4.4 SUV max value had SM2-3 tumors.

Figure 2

Prognostic analysis for survival curves in FDG uptake positive patients

A, The patients with SM2-3 tumors had poorer prognosis than those with M1-SM1 tumors.

B, The patients with poor differentiated tumors had poorer prognosis than those with well or moderately differentiated tumors.

Well ; well differentiated

Mode ; moderately differentiated

Poor ; poorly differntiated