^{18}F-fluorodeoxyglucose positron emission tomography predicts recurrence and histological grade of extrahepatic bile duct cancer

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Abstract. Malignant tumors in cholangiocarcinoma are diagnosed and staged using ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) and clinical analysis. However, comprehensive analysis, including pathological analysis, has not yet been sufficiently performed. In the present study, the maximum standardized uptake value (SUVmax) was calculated using FDG-PET and its relationship with clinicopathological factors was analyzed. The present study included 86 patients who underwent preoperative FDG-PET/computed tomography (CT) and did not receive chemotherapy among 331 patients with hilar and distal cholangiocarcinoma. Receiver operating characteristic analysis with recurrence events was used to determine the SUVmax cutoff of 4.9. Immunohistochemical staining of glucose transporter 1 (Glut1), hypoxia-inducible factor-1α and Ki-67 was performed for pathological analysis. The standardized uptake value (SUV)-high group (SUVmax ≥4.9) had a higher postoperative recurrence rate (P<0.046) and higher Glut1 and Ki-67 expression rates (P<0.05 and P<0.0001, respectively). Furthermore, SUVmax and Glut1 expression (r=0.298; P<0.01) and SUVmax and Ki-67 expression rates (r=0.527; P<0.0001) were positively correlated. The preoperative measurement of SUVmax by PET-CT is useful in predicting recurrence as well as cancer malignancy.

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Abbreviations: FDG-PET, 18F-fluorodeoxyglucose positron emission tomography; Glut1, glucose transporter 1; SUV, standardized uptake value; HIF-1α, hypoxia-inducible factor 1α; CT, computed tomography; OS, overall survival; DFS, disease-free survival

Key words: bile duct cancer, FDG-PET, Glut1, recurrence rate, SUV

Introduction

Bile duct cancer is a high-grade epithelial tumor that arises from the bile duct epithelium. This highly malignant tumor trait is caused due to difficulty in early diagnosis, the anatomical complication of radical resection and the insufficient efficacy of anticancer therapy. The 5-year survival rate for inoperable patients was ≤5% (1), and the overall 5-year survival rates of patients who undergo surgery are 20-30% after curative resection (2). The first choice for biliary tract cancer is surgical resection because its localization leads to better prognoses. Preoperative diagnosis, such as local extension and distant metastasis, is vital to decide cancer resection. Recently, the preoperative use of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) has been reported to be effective in detecting lymph node metastasis and unexpected distant metastasis on gallbladder carcinoma and biliary carcinoma with the advancement of diagnostic imaging (3-7). Additionally, various reports have demonstrated an association between the max value of standardized uptake value (SUVmax) and overall survival (OS) or disease-free survival (DFS) in the gallbladder and biliary tract cancers (7-12). However, only a few reports examine SUVmax values and histopathological characteristics. Paudyal *et al* (13) reported a correlation between F18-FDG and glucose uptake level by cholangiocarcinoma using immunohistochemistry in bile duct cancer. However, the investigation of the relationship between clinicopathological tumor grade and SUVmax value has not been fully clarified. Therefore, this study aimed to investigate the correlation between SUVmax and prognosis and recurrence in extrahepatic cholangiocarcinoma and analyze the correlation between SUVmax and tumor grade using immunostaining of proliferative capacity and glucose uptake of cancer cells.

Materials and methods

Patients and study design. This single-center retrospective study was approved by the Medical Ethics Committee of Hirosaki University Graduate School of Medicine. Of the 331 patients who underwent surgery for hilar and distal cholangiocarcinoma from January 2008 to May 2018, 86 patients who met the criteria were included in the control group. We

excluded patients with poorly controlled diabetes, patients with an inability to reduce obstructive jaundice who underwent semi-emergent surgery, and patients from whom informed consent for FDG-PET/CT examination could not be obtained. Furthermore, to eliminate the influence of inter-institutional errors in the SUVmax values, we excluded patients who had been examined preoperatively at other hospitals and did not undergo FDG-PET/CT at our hospital. Patients with liver metastasis, lung metastasis, bone metastasis, peritoneal dissemination, distant lymph node metastasis (para-aortic lymph node, extra-abdominal lymph node), or those who were inoperable because of their poor general condition were considered unsuitable for surgery and were treated with chemotherapy. The efficacy of preoperative chemotherapy in biliary tract cancer has not been established (14). Therefore, we did not administrate preoperative chemotherapy at our institution. All other patients considered to be operable (n=331) underwent surgery without preoperative chemotherapy.

Of these 86 patients, 58 were males and 28 were females, with a median age of 71 years. The locations of carcinoma were hilar (29 cases) and distal (57 cases) anatomically. Curative resection and lymph node dissection were performed depending on the primary tumor location, wherein pancreaticoduodenectomy or pylorus-preserving pancreaticoduodenectomy was performed in 56 cases, combined hepatectomy with bile duct resection in 26 cases and bile duct resection in 4 cases. After surgery, CT scans were performed every 3-6 months to identify any local recurrence or distant metastasis. Serum CA19-9, serum SPan-1, and serum DUPAN-2 levels were also periodically evaluated as tumor markers to aid in the diagnosis of recurrence. The definition of recurrence was confirmation of local or distant metastatic lesions on CT by a radiologist, accompanied by an increase of tumor markers. In particular, the following findings were used as criteria for local recurrence: i) soft tissue shadow with a tendency to enlarge near the resected primary tumor, ii) soft tissue shadow with deformation of the portal vein, hepatic artery, or hilar bile duct near the resected primary tumor, iii) FDG accumulation on FDG/PET-CT, and iv) soft tissue shadow that can be differentiated from an inflammatory mass.

Tegafur, gimeracil, and oteracil (S-1) at 80 mg/m^2 on days 1-14 every 3 weeks for 1 year were administered in 52 cases for adjuvant chemotherapy. Gemcitabine plus cisplatin to gemcitabine was administered in one patient and gemcitabine to cisplatin in another patient. Survival data were obtained from hospital medical charts. The median observation period was 31 months.

Data were retrospectively collected from an electronic medical records system and included the following: Information on clinical and demographic characteristics, pathological characteristics and stages, postoperative recurrence duration, and overall survival. All study procedures involving participants were performed following the ethical standards of the institutional and national research committees and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. None of the patients applied for withdrawal of consent.

Pathological analysis. All surgical specimens were fixed with 10% formalin at 25˚C for 48-72 h. In the pancreatoduodenectomy cases, the common bile ducts were sliced at right angles. The enlarged right and left lobectomy cases were resected at intervals of 5-7 mm intervals on a plane perpendicular to the long axis on the craniocaudal side. The extrahepatic bile duct resection cases were sliced in a plane perpendicular to the extrahepatic bile duct. Thinly sliced sections (4 μm) were stained with H&E (Hematoxylin 20 min and eosin 3 min at room temperature, usually 25˚C) for the histopathological examination. Clinicopathological findings, such as depth of tumor invasion, histological type, lymph node metastasis, lymphatic invasion, venous invasion, and stage, were reviewed according to the 8th edition of the Tumor-Node-Metastasis classification of the Union For International Cancer Control (15).

Immunohistochemical staining. Deparaffinized sections were immunohistochemically examined using the standard avidin-biotin-peroxidase complex method with an automated immunostainer (Benchmark XT; Ventana Medical System). In brief, deparaffinized slides were treated with Tris (pH: 7.8) at 95˚C for 44 min. The slides were treated with 5% non-fat dry milk at 37˚C for 15 min for blocking endogenous peroxides and protein. The slides were incubated with primary antibodies for 60 min at room temperature. The antibodies used were as follows: glucose transporter 1 (Glut1; rabbit polyclonal, Abcam, Catalog No: ab15309, dilution 1:200), hypoxia-inducible factor 1α (HIF-1α; Clone: H1α67, mouse monoclonal, MILLIPORE, Catalog No: MAB5382, dilution 1:500), and Ki-67 (Clone: MIB-1, mouse monoclonal, Dako, Catalog No: M7240, dilution 1:100). The Glut1 is a transmembrane protein and is associated with glucose transport inside and outside the cell. The HIF-1α is a typical hypoxia-related marker. The Ki-67 is a marker that determines the growth fraction of a given cell population.

Immunohistochemistry evaluation. Immunohistochemical staining specimens for Glut1, HIF-1α, and Ki-67 were evaluated by two experienced pathologists (TY and KH) without patient or clinical outcome information. The intensity of staining and the percentage of stained tumor cells were calculated for Glut1 and HIF-1α staining for all specimens. Additionally, the number of tumor cells with positive nuclei was calculated for Ki-67 staining. Specifically, the staining intensity of cells is classified into scores of 0 (negative), 1 (weak), 2 (intermediate), and 3 (strong) in the Glut1 and HIF-1α staining evaluation regarding the H score (range 0-300), and the percentage of stained tumor cells is shown in the total tumor cells (0-100%). The H score was then calculated by multiplying the staining intensity of tumor cells (0-3) by the distribution percentage of positive cells (0-100%). The Ki-67 ratio was calculated by counting the number of positive tumor cells per 1000 tumor cells in a tumor hot spot.

FDG-PET/CT and image analysis. All patients fasted for at least 4 h and water intake was encouraged to prepare them for FDG-PET/CT. F-18 FDG (FDG scan injectable, 185 MBq on the assay data; Nihon Medi-Physics), which was delivered via intravenous injection ~60 min before the initiation of scanning. Patients were advised to drink a sufficient amount of water and remain calm during the 60-min uptake period. Data in 7-8 bed positions with an acquisition time of 2.5-3.0 min per bed position were acquired using a FDG-PET/CT system (Discovery

ST Elite 16; GE Healthcare). The CT was performed first (30-80 mA, 120 kV, 3.75-3.27 mm slice thickness). The CT data were used for FDG-PET data attenuation correction as well as co-registration with the attenuation-corrected FDG-PET images. The PET data of the same body regions were immediately acquired following CT imaging. The FDG-PET, CT, and fused FDG-PET/CT images were available for review and were displayed in the axial, coronal, and sagittal planes on a viewer system (Discovery ST Elite 16; GE Healthcare). According to the previous study, SUVmax (g/ml) was evaluated in all histopathologically proven lesions (10). The SUVmax, which was defined as the highest SUV in the pixel with the maximal SUV within the region of interest, was measured and recorded for the focal areas of uptake. The SUVmax values were standardized for the injected dose and the patient's weight.

Statistical analysis. We used receiver operating characteristic (ROC) curves with postoperative recurrence as the categorical variable to calculate the cutoff value of the SUV_{max}. The Kaplan-Meier method was used for OS and the DFS analysis was used to estimate the event rates and the log-rank test for survival comparisons between patient groups. Univariate analysis was performed for prognostic factors using a log-rank test. Significant factors in univariate analysis were included in multivariate analysis using a Cox proportional hazards model. And Cox proportional analysis was used for multivariate analysis. The χ^2 and Fisher exact (categories with expected values <5) probability tests were used to examine the relationship between SUVmax and clinicopathological features, such as sex, age, location, macroscopic type, histology, lymphatic invasion, vessel invasion, perineural invasion, tumor status (T), node status (N), stage, portal vein invasion, resection margin status, preoperative biliary drainage and preoperative serum hemoglobin A1 (HbA1c). Continuous variables between two groups [SUVmax <4.9 (SUV-low) group and SUVmax ≥4.9 (SUV-high) group] were compared using the Mann-Whitney U test to examine the Glut1, HIF-1α and Ki-67 expressions. The association between the SUVmax and Glut1, HIF-1α and Ki-67 expressions was evaluated using Spearman's rank correlation test. The P-values of <0.05 was considered significant, and statistical analysis was performed using GraphPad Prism software version 9.0.

Results

Clinicopathological factors and SUVmax. From the ROC curve, the optimal cutoff value for SUV_{max} to predict recurrence was calculated to be 4.9 (area under the curve: 0.613) (Fig. 1). The median value was divided into groups of 44 cases with SUV-low and 42 cases with SUV-high. The comparison of each group with clinicopathological factors (sex, age, localization, macroscopic type, histology, lymphatic invasion, vessel invasion, perineural invasion, T status, N status, stage, portal vein invasion, resection status, preoperative biliary drainage, preoperative serum HbA1c, and cholangitis) showed no significant differences in any of the factors (Table I).

Pathological factors and SUVmax. The staining properties of Glut1 and HIF-1α in tumor cells between the SUV-low and SUV-high groups were compared using the H score, respectively. The Glut1 expression was higher in the SUV-high group than in the SUV-low group (P<0.05), and SUVmax and Glut1 (H score) showed a significant correlation (r=0.298, P<0.01). No correlation was obtained between the SUV-low and SUV-high groups for HIF-1α (H score). Additionally, no predominant correlation was found between the SUVmax and the HIF-1α (H score). The Ki-67 expression showed a significant difference between the SUV-low and SUV-high groups (P<0.0001). Similarly, a significant correlation was found between the SUVmax and the Ki-67 expression (r=0.527, P<0.0001). The comparison between Ki-67 expression and Glut1 (H scores) showed a significant correlation (r=0.252, P<0.05; Figs. 2 and 3).

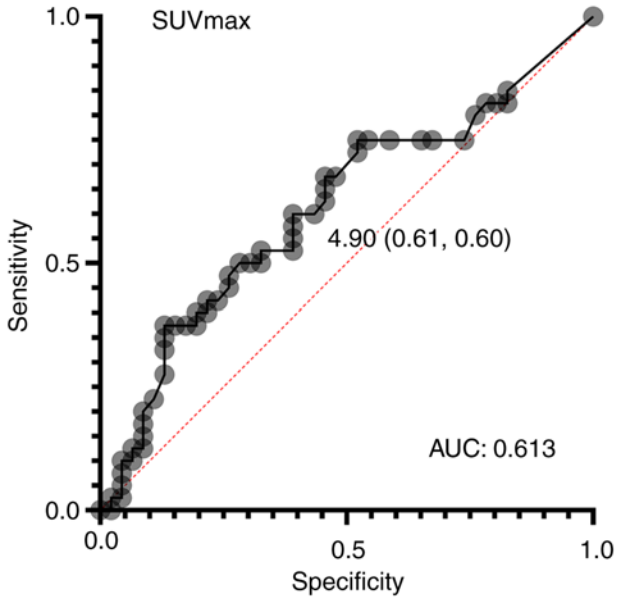

Figure 1. Receiver operating characteristic curve of the SUVmax for prediction of recurrence. The optimal cutoff value is 4.90. AUC, area under the curve; SUVmax, maximum standardized uptake value.

Clinicopathological features and OS and DFS. The Kaplan-Meier curves of OS and DFS for patients (SUV-low and SUV-high) are shown in Fig. 4. No significant difference was found between the SUVmax and OS, but the risk of recurrence was significantly higher in the SUVmax (≥4.9) group for DFS. The DFS data were analyzed for a total of 86 patients (SUV-low in 44 cases, SUV-high in 42 cases), and the results were summarized in Table II. Univariate analysis for DFS revealed tumor location (P=0.013), N status (P<0.001) stage (P<0.01), and SUVmax (P=0.046) and multiple analysis revealed tumor location (P=0.028) and N status (P<0.01) as clinicopathological factors. In multivariate analysis, the SUVmax was not an independent recurrence factor.

Cases of recurrence and characteristics of recurrence location. In the SUV-high and -low groups, there were 24 and 16 recurrence cases, respectively. The 24 SUV-high group recurrence cases included 11 (45.8%) local and 13 (54.2%) distant recurrence cases, whereas the 16 SUV-low group included 9 (56.3%) local and 7 (43.7%) distant recurrence cases, and there was no

Table I. Association between the SUV and clinicopathological factors.

Characteristics	SUVmax <4.9, n (n=44)	SUVmax ≥4.9, n (n=42)	P-value
Sex			
Male	31	27	0.65
Female	13	15	
Age, years			
≤70	17	21	0.40
>70	27	21	
Localisation			
Hilar	14	15	0.88
Distal	30	27	
Macroscopic type			
Nodular/papillary	21	18	0.09
Flat	23	24	
Histology			
Papillary/well	19	20	0.84
Moderately/poorly/ others	25	22	
Lymphatic invasion			
Absent	22	17	0.50
Present	22	25	
Vessel invasion			
Absent	24	21	0.84
Present	20	21	
Perineural invasion			
Absent	15	14	>0.99
Present	29	28	
pT status			
T1/T2	32	29	0.89
T3/T4	12	13	
pN status			
N0	32	22	0.08
N1/N2	12	20	
pStage			
I/II	34	31	0.90
III/IV	10	11	
Portal vein invasion			
Non-invasion	42	39	0.96
Invasion	2	3	
Resection margin			
Negative	42	37	0.39
Positive	2	5	
Preoperative biliary drainage			
Yes	29	33	0.29
No	15	9	
Preoperative serum HbA1c, %			
≤6.4	34	37	0.30
>6.4	10	5	
Cholangitis			
Absent	40	35	0.35
Present	4	7	

SUVmax, maximum standardized uptake value; pT, pathological T; pN, pathological N; pStage, pathological Stage; HbA1c, hemoglobin A1c.

significant difference in recurrence between the SUV-high and -low groups (Table III).

Pathological characteristics of recurrence and non-recurrence cases in the SUVmax low and SUVmax high groups. The comparison of the clinicopathological factors (localization, histology, lymphatic invasion, vessel invasion, perineural invasion, T status, N status, stage, portal vein invasion, and resection status) between the recurrence and non-recurrence subgroups in the SUV_{max} low and SUV_{max} high groups showed no significant differences in any of the factors (Tables SI and SII).

Discussion

Our study results revealed two points. First, the SUV-high group had a higher postoperative recurrence rate than the SUV-low group. Second, the SUV-high group showed higher Glut1 and Ki-67expression rates. Furthermore, increased SUVmax, Glut1, and Ki-67 expression rates were positively correlated, and the Ki-67 expression rate was positively correlated with Glut1 expression. The relationship between SUVmax and prognosis and recurrence prediction of cholangiocarcinoma was previously clarified using FDG-PET/CT. The prognosis and recurrence rate was reported to worsen in the SUV-high group (8,9). Our study found no significant difference in prognosis but a significantly higher recurrence rate in the SUV-high group. The results may differ because the previous reports include surgically unresectable cases (8), gallbladder cancer, and intrahepatic cholangiocarcinoma, which are different from our study in terms of the target cases (9). Furthermore, the median SUVmax of cholangiocarcinoma is reported to vary depending on the primary tumor, wherein intrahepatic cholangiocarcinoma has the highest SUVmax, followed by gallbladder carcinoma and extrahepatic cholangiocarcinoma (3). Our study only focused on patients with resected extrahepatic cholangiocarcinoma. Hence, the results may have differed due to control case differences. Therefore, our study, which focused on surgically resected extrahepatic cholangiocarcinoma cases, was judged useful in extracting a high recurrence group.

Furthermore, the SUV-high group correlated with the histological malignancy of the tumor using immunostaining

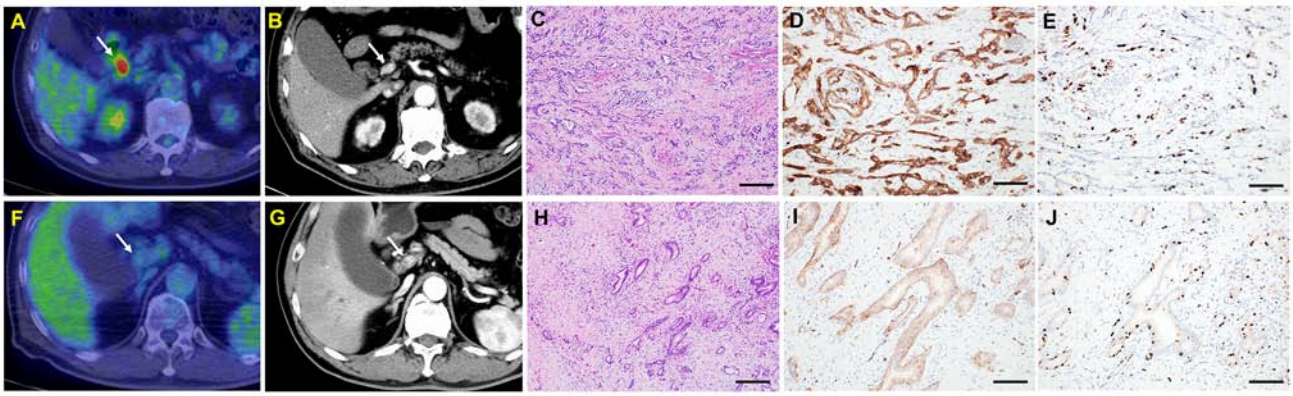

Figure 2. Representative bile duct cancer cases in the (A-E) SUV-high (≥4.9) and (F-J) SUV-low (<4.9) groups. (A) FDG/PET-CT image of distal cholangiocarcinoma (SUVmax <4.9). High absorption of SUVmax (13.1) was observed in the distal bile duct. (B) Contrast-enhanced CT image. Arrows indicate the bile duct cancer lesion. The distal bile duct exhibited wall thickening with increased contrast density. (C) H&E image. Moderate to poorly differentiated tumor cells were proliferating (Scale bar, 200 μm). (D) Glut1 immunohistochemistry. Strong expression of Glut1 of tumor cells (score 3) was observed (Scale bar, 100 μm). (E) Ki-67 immunohistochemistry. The Ki-67 expression rate was 40% (Scale bar, 100 μm). (F) PET-CT image of distal cholangiocarcinoma (SUVmax <4.9). In this tumor lesion, the SUVmax was low (2.8). (G) Contrast-enhanced CT image. Arrows indicate the bile duct cancer lesion. (H) H&E image. Well-to-moderately differentiated tumor cells were proliferating (Scale bar, 200 μm). (I) Glut-1 immunohistochemistry. Weak expression of Glut1 of tumor cells (score 1) is shown (Scale bar, 100 μm). (J) Ki-67 image. The Ki-67 expression rate was 10% (Scale bar, 100 μm). Glut1, glucose transporter 1; PET-CT, positron emission tomography-computed tomography; SUV, standardized uptake value; SUVmax, maximum standardized uptake value.

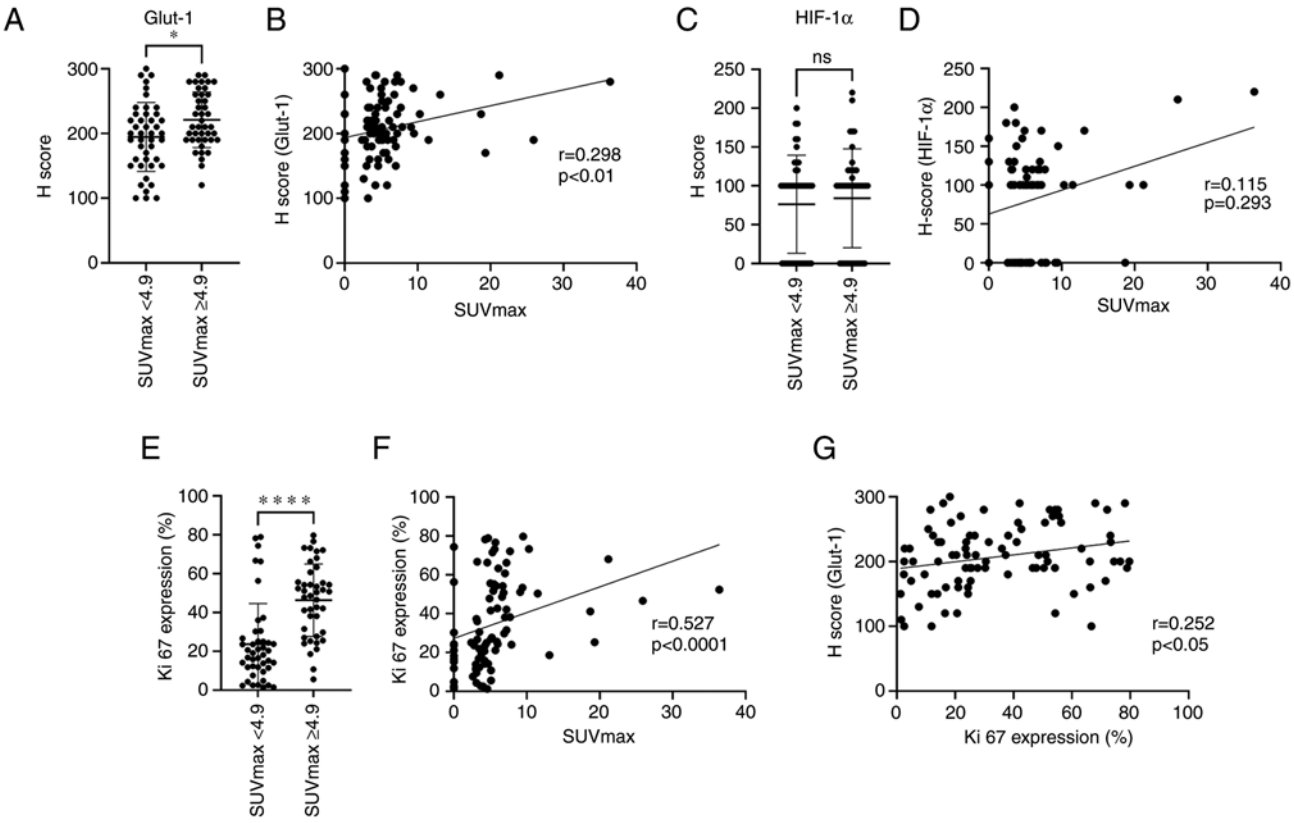

Figure 3. SUVmax vs. immunostaining results. (A) SUVmax vs. Glut1 (H score). The SUV-high (≥4.9) group showed predominantly elevated Glut1 (H score) expression (*P<0.05). (B) Significant correlation between SUVmax and Glut1 (H score). (C) SUVmax vs. HIF-1α (H score). No association was observed between SUVmax (SUVmax <4.9; SUVmax ≥4.9) and HIF-1α. (D) No correlation was observed between SUVmax and increased HIF-1α values. (E) SUVmax vs. Ki-67 expression (%). The SUV-high (≥4.9) group exhibited predominantly elevated Ki-67 expression (%; (****P<0.00001). (F) There was a significant positive correlation between SUVmax and Ki-67 expression (%). (G) There was a significant positive correlation between Ki-67 expression (%) and Glut1 (H score). Glut1, glucose transporter 1; HIF-1α, hypoxia-inducible factor 1α; ns, not significant; SUVmax, maximum standardized uptake value.

in resection specimens. First, tumor cells in the SUV-high group predominantly expressed high Glut1 levels. The FDG, which is used in FDG-PET/CT, is taken up into cells through glucose transporters (16). Previous studies have revealed that increased Glut1expression in cholangiocarcinoma correlates with decreased tumor differentiation, increased lymphatic invasion, and increased perineural invasion and is a poor prognostic factor, which indicates a correlation between Glut1 expression

Table II. Univariate and multivariate analyses of the DFS.

		Univariate analysis		Multivariate analysis	
Characteristics	No.	Median DFS, months	P-value	Hazard ratio (95% CI)	P-value
Age, years					
≤70	38	39.2	0.84		
>70	48	40.2			
Localisation					
Hilar	29	18.9	0.01	2.27 (1.09-4.71)	0.03
Distal	57	N/A			
Macroscopic type					
Nodular/papillary	49	33.4	0.09		
Flat	37	N/A			
Histology					
Papillary/well	39	N/A	0.21		
Moderately/poorly/others	47	34.1			
Lymphatic invasion					
Absent	39	N/A	0.09		
Present	47	34.1			
Vessel invasion					
Absent	45	49.5	0.14		
Present	41	27.3			
Perineural invasion					
Absent	29	N/A	0.08		
Present	57	29.1			
pT status					
T1/T2	61	39.4	0.58		
T3/T4	25	40.2			
pN status					
N0	54	N/A	<0.001	3.21 (1.36-7.60)	<0.01
N1/N2	32	18.2			
pStage					
I/II	65	N/A	<0.01	0.79 (0.29-2.14)	0.65
III/IV	21	18.2			
Portal vein invasion					
Non invasion	81	39.4	0.22		
Invasion	5	N/A			
Resection margin					
Negative	79	40.2	0.36		
Positive	7	18.9			
Adjuvant chemotherapy					
No	34	33.4	0.62		
Yes	52	44.1			
SUVmax					
<4.9	44	N/A	<0.05	1.77 (0.93-3.35)	0.08
≥4.9	42	25.1			

DFS, disease-free survival; SUVmax, maximum standardized uptake value; pT, pathological T; pN, pathological N; pStage, pathological Stage; HbA1c, hemoglobin A1c; N/A, not applicable (median DFS not reached).

Table III. Cases of recurrence and characteristics of recurrence locations.

		Recurrence site	
SUVmax	Cases	Local, n (%)	Distant, n (%)
≥4.9	24	11 (45.8)	13 (54.2)
<4.9	16	9 (56.3)	7 (43.7)

SUVmax, maximum standardized uptake value.

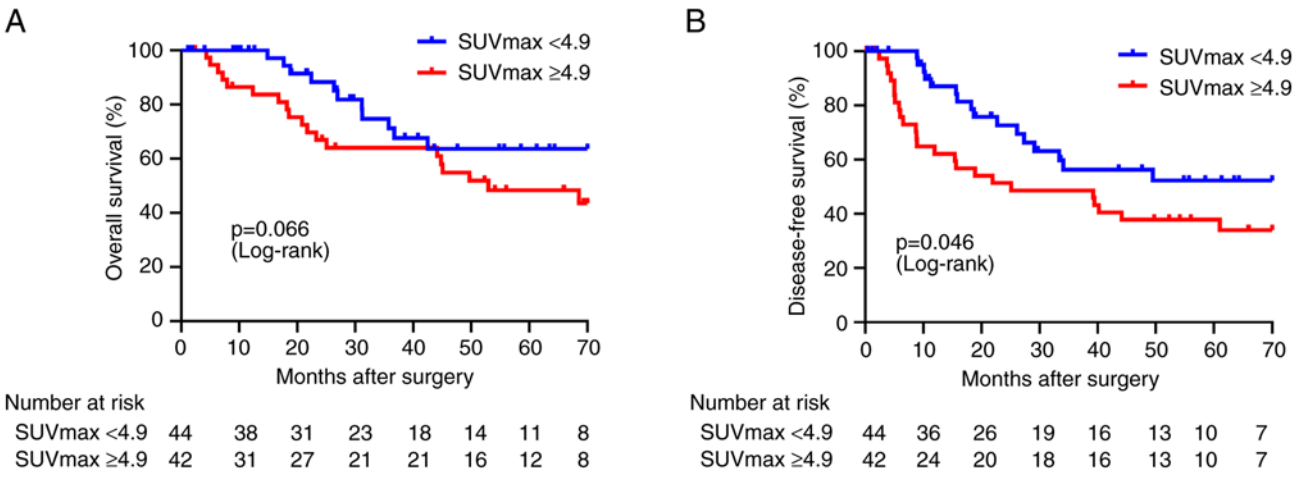

Figure 4. Kaplan-Meier overall survival and disease-free survival curves for the patients (SUVmax <4.9 and SUVmax ≥4.9). (A) No significant association was observed between SUVmax (SUVmax <4.9 and SUVmax ≥4.9) and overall survival (P=0.066). (B) The SUV-high group (SUVmax ≥4.9) had a significantly lower disease-free survival rate (P=0.046). SUVmax, maximum standardized uptake value.

and cholangiocarcinoma malignancy (17,18). Paudyal *et al* (13) studied FDG uptake and Glut1 expression in cholangiocarcinoma, reported a positive correlation between FDG uptake and Glut expression, and noted that increased FDG uptake correlated with decreased tumor differentiation. Our study found no significant difference between the SUVmax and tumor differentiation; however, Ki-67 expression, which is a marker of cell proliferative potential, was evaluated as tumor malignancy and was significantly increased in the SUV-high group. Particularly, the results are similar to previous reports in correlation with tumor grade. Cancer cells require more glucose than normal cells, and high-grade tumor cells with high proliferative potential have a rapid cell cycle and increased cell proliferation. This study points to the possibility of assessing the trend of cell proliferation by evaluating the SUVmax values. Then, we examined the hypoxia marker HIF-1α and revealed no correlation between FDG uptake and HIF-1α in cancer cells. The HIF-1α is a transcription factor that is expressed under hypoxic conditions and is not degraded and stabilized when cells are exposed to hypoxia, resulting in rapid HIF-1α accumulation. Thus, HIF-1α has been reported to induce various growth factor transcription, such as vascular endothelial growth factor and Glut1 (19-21). Particularly, a positive correlation was assumed between increased expression of SUVmax and Glut1 and HIF-1α, but the study results revealed no significant effects. The relationship between HIF-1α and FDG-PET/CT parameters is controversial. Kaymak *et al* (22) revealed no significant association between HIF-1α and SUVmax in colorectal carcinoma probably because of the heterogeneity of the oxygen situation within the tumor. The tumor invasion area is assumed to be more hypoxic than the bile duct surface. The HIF-1α expression evaluation throughout cancer may have been affected, which is an issue for future study.

This study had two limitations. First, this study was retrospectively performed not as a randomized controlled study and included a relatively small number of patients who underwent FDG-PET/CT. Furthermore, only patients who had undergone FDG-PET/CT at our hospital were included in the study, but the criteria for inclusion at our hospital were not clear. Additionally, hilar and distal cholangiocarcinoma were analyzed together. However, the hilar and distal cholangiocarcinoma have different tumor characteristics, which possibly affected the OS. Therefore, preoperative testing, including FDG-PET/CT, is currently being performed at our hospital for patients with cholangiocarcinoma, and we plan to accumulate and analyze more cases in the future. Second, we only focused on cancer cells and performed immunostaining evaluation for cancer malignancy. However, cancer malignancy involves cancer cells and the cancer microenvironment, including the stroma and immune cells. Therefore, a future comprehensive study that includes the cancer microenvironment is expected.

In conclusion, preoperative measurement of SUVmax by FDG/PET-CT is useful in predicting recurrence as well as cancer malignancy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TYa, TYo, HS, SM, SG and NK designed the experiments. TYa and TYo performed the experiments. TYa, TYo, KI, HK and KH performed the data analysis. TYa and TYo wrote the main manuscript text, and prepared the figures. HS evaluated the radiological images. TYo, SG and HK contributed to histological evaluation. SM, KI and KH provided clinical information including adjuvant chemotherapy information. KI, HK and KH critically revised the manuscript. TYo and HK confirm the authenticity of all the raw data. All authors agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The research protocol was approved by the ethics committee of Hirosaki University (2022-038; Hirosaki, Japan). All study procedures involving human participants were performed following the ethical standards of the institutional and/or national research committee and in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients provided written informed consent to participate.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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