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ORIGINAL ARTICLE

Success rate of microsatellite instability examination and complete response with pembrolizumab in biliary tract cancer

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Key words
biliary tract neoplasms, cholangiocarcinoma, endoscopic ultrasound-guided fine-needle aspiration, microsatellite instability, pembrolizumab.

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Abstract

Background and Aim: The success rate of microsatellite instability (MSI) examination in biliary tract cancer (BTC) and the treatment outcomes of pembrolizumab in patients with MSI-high (MSI-H) BTC have not been fully investigated. We examined the success rate of MSI examination and the rate of MSI-H status in patients with BTC as well as the treatment outcomes of patients with MSI-H status who underwent pembrolizumab treatment.

Methods: We retrospectively reviewed 60 consecutive patients with unresectable or postoperative recurrent BTC who underwent MSI examination in a Japanese cancer referral center between January 2019 and September 2020.

Results: The study included 24 intrahepatic cholangiocarcinomas, 12 hilar cholangiocarcinomas, 4 distal cholangiocarcinomas, 16 gallbladder carcinomas, and 4 ampullary carcinomas. The methods of cancer tissue sampling were percutaneous liver tumor biopsy in 26 cases, surgery in 15 cases, endoscopic ultrasound fine-needle aspiration in 12 cases, transpapillary bile duct biopsy in 5 cases, and others in 2 cases. The success rate of MSI examination was 98.3% (59 of 60). MSI examination failed in only one case using a surgical specimen due to time-dependent degradation of DNA. The frequency of MSI-H BTC was 3.3% (2 of 60 cases). One patient with MSI-H intrahepatic cholangiocarcinoma achieved a complete response with pembrolizumab treatment.

Conclusions: MSI examinations in BTC were successful in almost all cases, regardless of tissue sampling methods. We experienced a case in which pembrolizumab resulted in a complete response to MSI-H BTC. Since pembrolizumab for MSI-H BTC could prolong survival time, MSI examination should be performed proactively to increase treatment options.

Introduction

Patients with biliary tract cancer (BTC), including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and ampullary carcinoma, are frequently diagnosed at an advanced stage and have a poor prognosis.[1,2,3,4] One of the reasons for the dismal prognosis of advanced BTC is that efficient systemic chemotherapy is limited.[5,6] Gemcitabine plus cisplatin (GC) combination chemotherapy is the standard first-line regimen for BTC.[7,8,9] Recently, gemcitabine plus oral fluoropyrimidine S-1 therapy has been proven to be non-inferior to GC therapy.[10] Furthermore, GC plus S-1 therapy has shown superiority over GC in a phase III trial.[11] However, the choice of chemotherapeutic drugs for BTC remains limited. In addition, optimal second-line chemotherapy has not yet been established.[12]

Pembrolizumab is a humanized monoclonal antibody against programmed cell death 1 (PD-1) protein that activates a host immune response against tumors that express programmed cell death ligand 1 (PD-L1).[13] In patients with microsatellite instability-high (MSI-H)/mismatch repair-deficient (dMMR) solid tumors, the antitumor effect of pembrolizumab has been demonstrated in clinical trials.[14,15] A 34.3% objective response rate has been reported for pembrolizumab in previously treated unresectable or metastatic MSI-H non-colorectal cancer.[15] Consequently, pembrolizumab has been approved in many countries for the treatment of advanced solid MSI-H tumors. In December 2018, pembrolizumab was approved in Japan for the treatment of unresectable or metastatic MSI-H solid tumors that continue to progress after standard chemotherapy.[16,17] However, there have been few reports on the success rates of MSI examination in BTC and treatment with pembrolizumab for MSI-H BTC.

Particularly, to our knowledge, there are no reports on the feasibility of MSI examination using small samples collected by endoscopic ultrasound fine-needle aspiration (EUS-FNA), liver tumor biopsy, or bile duct forceps biopsy in patients with BTC.

In this single-center retrospective study, we examined details of specimen collection methods, the success rate of MSI examinations, and the rate of MSI-H status in patients with BTC. Similarly, we described the details of patients with MSI-H status who underwent pembrolizumab treatment, including a case in which complete response was achieved after pembrolizumab administration.

Methods

We retrospectively reviewed the clinical data of 60 patients with unresectable or postoperative recurrent BTC, including intrahepatic cholangiocarcinoma, who underwent MSI examination between January 2019 and September 2020 at a Japanese cancer referral center (Osaka International Cancer Institute). Patients with BTC who did not undergo MSI examination were excluded from this study. Diagnoses of malignancy were pathologically proven, and MSI examinations were performed with the MSI Kit (FALCO Biosystems, Kyoto, Japan) in all cases. For each patient, data were collected regarding age, sex, location of the primary tumor, histological type, the extent of disease (unresectable disease/recurrence after surgery), methods of cancer tissue sampling, and the period from tissue sampling to MSI examination. In the patients whose samples were obtained by EUS-FNA, data on the target of FNA, targeted tumor size, needle gauge, and the number of punctures were additionally collected. This study was approved by the Institutional Review Board of the Osaka International Cancer Institute (20148) and performed in accordance with the Declaration of Helsinki.

Results

Sixty consecutive patients with unresectable or postoperative recurrent BTC were analyzed in this study, including 24 intrahepatic cholangiocarcinomas, 12 hilar cholangiocarcinomas, 4 distal cholangiocarcinomas, 16 gallbladder carcinomas, and 4 ampullary carcinomas. The patient characteristics are summarized in Table 1. Thirty-one patients (51.7%) were men; the median age of the study patients was 67 years (range, 30–83 years). The histological types were adenocarcinoma in 59 patients and adenosquamous carcinoma in 1 patient. The methods of cancer tissue sampling were as follows: percutaneous liver tumor biopsy in 26 cases, surgery in

Table 1 Patient characteristics ($n = 60$)

Clinical characteristics of the study patients	
Sex	
Male	31 (51.7%)
Female	29 (48.3%)
Median age (years)	67 (range: 30–83)
Location of primary tumor	
Intrahepatic	24 (40.0%)
Extrahepatic-perihilar	12 (20.0%)
Extrahepatic-distal	4 (6.7%)
Gallbladder	16 (26.7%)
Ampulla of Vater	4 (6.7%)
Histological type	
Adenocarcinoma	59 (98.3%)
Adenosquamous carcinoma	1 (1.7%)
Extent of disease	
Unresectable	45 (75.0%)
Recurrence after surgery	15 (25.0%)
Methods of cancer tissue sampling	
Percutaneous liver tumor biopsy	26 (43.3%)
Surgery	15 (25.0%)
EUS-FNA	12 (20.0%)
Transpapillary bile duct biopsy	5 (8.3%)
Endoscopic forceps biopsy of ampulla of Vater	1 (1.7%)
CT-guided lung biopsy	1 (1.7%)
Median duration from sampling to MSI examination (days)	70 (range 3–2135)

CT, computed tomography; EUS-FNA, endoscopic ultrasound fine-needle aspiration; MSI, microsatellite instability.

Table 2 Cases of endoscopic ultrasound fine-needle aspiration (EUS-FNA)

Case	Sex	Age (years)	Primary tumor lesion	Target of FNA	Target size (mm)	Needle gauge	Needle shape	Number of punctures
1	Male	53	Intrahepatic	LN	35	22	Lancet	3
2	Female	71	Intrahepatic	Liver	85	22	Lancet	4
3	Male	62	Intrahepatic	LN	28	22	Lancet	4
4	Male	63	Perihilar	LN	12	22	Lancet	2
5	Male	65	Perihilar	LN	32	20	Reverse-bevel	7
6	Female	70	Gallbladder	LN	35	22	Lancet	2
7	Female	57	Gallbladder	LN	25	22	Lancet	2
8	Female	69	Gallbladder	LN	30	22	Lancet	2
9	Male	67	Gallbladder	LN	10	22	Lancet	3
10	Female	49	Gallbladder	LN	69	22	Lancet	1
11	Male	56	Gallbladder	LN	20	22	Lancet	1
12	Female	72	Gallbladder	LN	17	22	Lancet	2

LN, lymph node.

15 cases, EUS-FNA in 12 cases, transpapillary bile duct biopsy in 5 cases, endoscopic forceps biopsy of the ampulla of Vater in 1 case, and computed tomography (CT)-guided lung biopsy in 1 case. The median duration from cancer tissue sampling to MSI examination was 70 days (range, 3–2135 days).

Table 3 Characteristics of patients with microsatellite instability (MSI)-high status

	MSI-high case A	MSI-high case B
Sex	Male	Male
Age (years)	71	70
Primary tumor lesion	Intrahepatic carcinoma	Distal bile duct
Histology	Adenocarcinoma	Adenocarcinoma
Extent of disease	Recurrence after surgery	Recurrence after surgery
Methods of cancer tissue sampling	Surgery	Surgery
Period from sampling to MSI examination (days)	329	1025
Administration of pembrolizumab	Done	No administration

Among the 12 patients who underwent EUS-FNA, the biopsy targets were enlarged lymph nodes in 11 cases and intrahepatic tumors in 1 case (Table 2). The median size of the targeted tumors was 29 mm (range, 10–85 mm). The needles used for EUS-FNA were 22 gauge (lancet needle) and 20 gauge (reverse-bevel needle) in 11 cases and 1 case, respectively. The median number of punctures was 2 (range, 1–7), and no complications were observed.

Overall, MSI examinations were successful in 98.3% (59 of 60) of the cases. In all cases where biopsy specimens and FNA specimens were used, MSI examinations were successfully performed. MSI examination failed in only one case (1.7%) due to the time-dependent degradation of DNA. In this case, an old surgical specimen of hilar cholangiocarcinoma was submitted for MSI examination (duration from sampling to MSI examination: 5.8 years).

MSI-H status was observed in 3.3% (2 of 60) of cases (Table 3). One patient with recurrence after surgery for distal cholangiocarcinoma (case B) had a fistula between the recurrent tumor and the colon in the abdominal cavity, and the infection could not be controlled with conservative antibiotic therapy and endoscopic drainage. Surgical operation with colostomy was proposed; however, the patient did not wish to undergo surgery and chose the best supportive care instead. Therefore, the patient

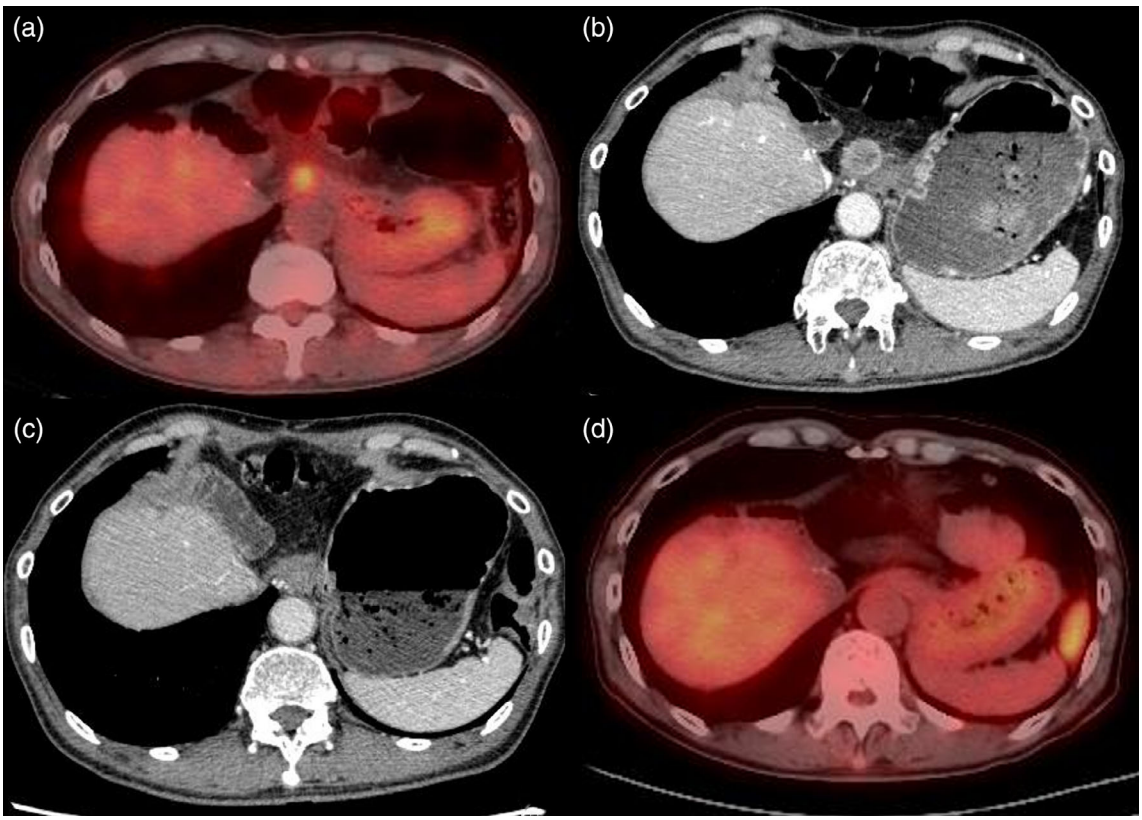

Figure 1 (a) Positron emission tomography/computed tomography (PET/CT) scan showing fluorodeoxyglucose (^{18}F-FDG) uptake in a 12-mm lymph node (LN) adjacent to the gastroesophageal junction before first-line chemotherapy (gemcitabine and cisplatin). (b) Contrast-enhanced CT (CECT) showing further enlargement of the LN to 21 mm after 3 months with first-line chemotherapy. (c) CECT showing the disappearance of the enlarged LN after three cycles of pembrolizumab. (d) PET/CT scan showing the disappearance of ^{18}F-FDG uptake after seven cycles of pembrolizumab.

(case B) did not undergo pembrolizumab treatment. Another patient with recurrence after surgery for intrahepatic cholangiocarcinoma (case A) underwent pembrolizumab treatment. This patient was a man in his 70s who underwent left hepatic trisegmentectomy for intrahepatic cholangiocarcinoma with Union for International Cancer Control staging pT3N1M0. Seven months after surgery, contrast-enhanced CT (CECT) revealed the recurrence of a 12-mm lymph node adjacent to the gastroesophageal junction where fluorodeoxyglucose (^{18}F-FDG) uptake was observed with a standardized uptake value of 3.2 on the positron emission tomography/CT (PET/CT) scan (Fig. 1a). The enlarged lymph node was histologically diagnosed as adenocarcinoma using EUS-FNA. After 3 months of first-line chemotherapy (gemcitabine and cisplatin), the disease was judged to have progressed due to further enlargement of the lymph node to 21 mm on CECT (Fig. 1b). Tumor markers were within the normal range; however, carbohydrate antigen 19-9 (CA19-9) levels gradually increased (37 U/mL). Since the MSI-H status was confirmed in the resected specimen, treatment with pembrolizumab was initiated (200 mg, triweekly). The patient tolerated treatment with a mild hyperthyroid abnormality. After 3 cycles, a complete response was achieved with the disappearance of the enlarged lymph node on CECT (Fig. 1c). The serum CA19-9 level decreased rapidly to <2 U/mL. ^{18}F-FDG uptake also disappeared from the PET/CT scan after 7 cycles (Fig. 1d). The patient has been maintaining complete response for 1 year and 10 months since the initiation of pembrolizumab, which continues to be administered.

Discussion

Accurate and timely repair of DNA is essential for genetic stability and preventing the transformation of normal cells into cancer cells.[18] Deficiencies in MMR pathways lead to MSI-H status and result in many mutations that encode tumor neoantigens.[19] Therefore, MSI-H/dMMR cancers are likely to be immunogenic and have the potential to be sensitive to immune checkpoint inhibitors.[20] MSI examination requires histological specimens. Compared with other gastrointestinal cancers, such as gastric cancer and colorectal cancer, MSI examinations in BTC have two concerns: first, the difficulty in collecting histological specimens, especially in unresectable cases, and second, the small volume of specimens obtained by biopsies. Due to these concerns, there have been a few reports on MSI examination in BTC in clinical practice. To our knowledge, this is the first report revealing that MSI could be successfully performed in patients with BTC not only with surgical specimens but also with small specimens collected using methods such as EUS-FNA, liver tumor biopsy, and bile duct forceps biopsy.

In this study, we demonstrated a high success rate for MSI examination (98.3%). We previously reported that the success rate of MSI examination in pancreatic cancer was 99.5% (183 of 184).[21] In BTC, as in pancreatic cancers, MSI examination was shown to be feasible with a very high probability. Among the 60 patients with BTC in this study, tissues from needle biopsies or endoscopic forceps biopsies were used in 75.0% (45 of 60) of the cases, whereas surgical specimens were used in only 25.0% (15 of 60). MSI examination was successful in all cases using a small amount of tissue collected by needle biopsy, including 12 EUS-FNA cases. EUS-FNA is considered to be a safe method of examination in these cases.[22,23] In the present study, the EUS-FNA procedures, such as the number of punctures and needle gauge, were conventional, and no EUS-FNA-related complications were observed. Targeted lesions of EUS-FNA were mostly metastatic lymph nodes, suggesting the importance of obtaining histological samples from enlarged lymph nodes by EUS-FNA even when it is difficult to collect histological samples from the primary tumor.

In the only case in which MSI examination failed, an old surgical specimen was used (duration from sampling to MSI examination: 5.8 years). It has been reported that specimens for genome sequencing should be used within 3 years because the nucleic acid quality of formalin-fixed paraffin-embedded tissues deteriorates over time.[24] Importantly, if too much time has passed since the tissue sampling, MSI examination may not be possible due to DNA degradation.

Since advanced BTC is a disease with a poor prognosis and limited chemotherapy options available, pembrolizumab as a new treatment option for BTC is attracting attention. Pembrolizumab has been proven to be efficient in MSI-H/dMMR solid cancers regardless of the primary site.[14,15] While a high response rate has been reported in MSI-H BTC,[15] the rate of BTC with MSI-H status is low. In this study, the frequency of -MSI-H status in BTC was 3.3% (2 of 60), consistent with previous reports (1–3%).[14,25] Of the two MSI-H patients in this study, one patient underwent pembrolizumab therapy. Following postoperative lymph node recurrence after surgery for intrahepatic cholangiocarcinoma, the patient experienced a complete response with pembrolizumab. This dramatic response to pembrolizumab highlights the importance of MSI examination in BTC. Although the rate of BTC with MSI-H status is low, pembrolizumab offers a promising treatment strategy for unresectable or recurrent MSI-H BTC.

The present study had several limitations. This was a retrospective study conducted at a single referral center with a limited number of patients. Because of the rarity of patients with MSI-H BTC, the treatment outcomes of these patients should be examined on a large scale.

In conclusion, MSI examination in BTC was successful in almost all cases, regardless of the tissue sampling method. The frequency of MSI-H BTC at our institution was 3.3%. Since pembrolizumab for MSI-H BTC could prolong survival time, MSI examination should be performed proactively to increase treatment options.

Data availability statement. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy issues.

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