Original paper

The prognostic role of epidermal growth factor receptor mutation in completely resected ampullary adenocarcinoma

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The aim of this study is to make a differential diagnosis and prognosis of the ampullary adenocarcinoma subtypes. We also investigated the role of prognostic markers PD-1 and PD-L1, and epidermal growth factor receptor (EGFR).
Local or locally advanced stage ampullary adenocarcinoma patients who had undergone pancreaticoduodenectomy at the time of diagnosis were included. MUC1, MUC2, MUC5AC, CDX2, CK7, CK20, PD-1, and PDL-1 were analysed immunohistochemically, and EGFR was analysed by real-time polymerase chain reaction. According to histopathological and immunohistochemical evaluation, we found 27 patients as pancreatobiliary type and 56 patients as intestinal type adenocarcinoma. The median survival of patients with intestinal and pancreatobiliary type adenocarcinoma was 23 months and 76 months ($p = 0.201$), respectively.
When the survival of PD1-positive ($n = 23$) and PD-L1-positive ($n = 18$) patients were compared with the patients with negative staining ($n = 60$, $n = 65$), no significant difference was found. Epidermal growth factor receptor mutation was detected in a total of 6 patients, and 5 of these 6 mutations were shown in intestinal type tumours and one in a pancreatobiliary type tumour. A significant difference was determined in terms of overall survival for the patients with EGFR mutations compared to those without ($p = 0.008$). In conclusion, we could reveal the prognostic significance of EGFR mutation, which is also a target molecule.

Key words: ampullary cancer, EGFR, intestinal, pancreatobiliary, prognosis.

Introduction

Ampullary tumours are rare among gastrointestinal system malignancies. Ampullary adenocarcinoma, which is the dominant type of ampullary tumour, is divided into 2 different biological subtypes, intestinal and pancreatobiliary, according to its histological features and immunohistochemical (IHC) staining. To make this distinction, many molecular markers and pathways have been evaluated. In pancreatobiliary type, cytokeratin 7 (CK7), mucin 1 (MUC1), mucin 5AC (MUC5AC), AKT-MAPK pathway, RTK-RAS

signalling, and TP53-Rb signalling are frequently detected. In intestinal type, cytokeratin 20 (CK20), mucin 2 (MUC2), caudal type homeobox 2 (CDX2), cyclooxygenase-2 (COX-2), carcinoembryonic antigen (CEA), E-cadherin, and β-catenin-Wnt pathway are observed more often [1–7].

Difficulties may be encountered in differentiating adenocarcinomas of the ampulla into different subtypes, especially in large tumours. In the literature there are many studies with conflicting results about the frequency and prognosis of intestinal and pancreatobiliary type ampullary adenocarcinoma. In some studies, it was concluded that the pancreatobiliary phenotype was more frequently found and had a significantly worse prognosis than those with the intestinal phenotype [3]. On the other hand, other studies found the opposite or no significant difference in terms of both the frequency and the survival outcome between intestinal and pancreatobiliary subtypes of ampullary cancer [4–7]. Therefore, it is of great importance to clearly distinguish these subtypes to find out both their prognostic importance and different approaches in their treatment.

The only potentially curative treatment for ampullary carcinoma is surgical resection. Complete resection with negative surgical margins is a prerequisite for cure of cancer. The prognosis of resected ampullary cancer also depends on the depth of local invasion and the presence of nodal metastases [8–13]. Despite potentially curative resections, more than half of ampullary cancer patients die from recurrent disease. This suggests the need for effective adjuvant therapy. Many clinicians do not recommend adjuvant treatment for resected ampullary cancers, citing the lack of data from randomized trials. However, there are also clinicians who suggest that these patients should be managed in a manner similar to that used for operable pancreatic cancer instead of intestinal cancer. Additionally, in metastatic disease, it is also unclear which systemic treatments are more effective, and the role of immunotherapies and targeted therapies is not known either. So, the optimal treatment strategy for primary ampullary carcinoma both at an early stage and in the metastatic setting is not clear as well as whether histological subtypes can be used to personalize treatment decisions.

The aim of this study is to make the differential diagnosis of the histological subtype of the ampullary adenocarcinoma by histopathological and IHC methods, to investigate the prognostic importance of these subtypes and other molecules like PD-1, PD-L1, or epidermal growth factor receptor (EGFR), and also to evaluate the effectiveness of the chemotherapy protocols used in the treatment of ampullary cancer.

Material and methods

Patient selection

In this study, patients with ampullary cancer, who were followed up and treated in our Medical Oncology Clinic between 2006–2016 were investigated. Ampullary adenocarcinoma patients with local or locally advanced disease who had undergone curative resection at the time of diagnosis were included in the study, while non-operated patients ($n = 5$) or patients with metastasis ($n = 3$) were excluded. All the patients had had the Whipple procedure (pancreaticoduodenectomy). Three patients who had endoscopic resection only were not included in the study. In addition, cases ($n = 28$) who died within 2 months of pancreaticoduodenectomy were also excluded. Paraffin blocks of these patients were obtained, and real-time polymerase chain reaction (RT-PCR) for EGFR and IHC analysis for CK7, CK20, MUC1, MUC2, MUC5AC, CDX2, PD-1, and PD-L1 were performed. Informed consent was obtained from all the participants, and the institution's Ethics Committee approved the study.

Histopathological evaluation

The cases were evaluated together with their macroscopic and microscopic features as well as their clinical findings and reports of endoscopic imaging and other imaging methods, and they were regrouped according to the World Health Organization 2019 ampullary tumours classification system. During microscopic examination pathologic stage, histologic grade, tumour invasion, lymphovascular invasion, perineural invasion, and lymph node involvement were re-evaluated. Then, histological subtypes of ampullary adenocarcinomas were determined primarily by direct microscopic examination with haematoxylin-eosin stain. Afterwards, the recommendations of the College of American Pathologists (CAP) protocol were applied to classify the tumours' IHC [14]. According to the recommended study of Ang *et al*. triple system, intestinal type tumours are typically positive for CK20 or CDX2 or MUC2 with negative for MUC1, or they are positive for CK20, CDX2, and MUC2, irrespective of the MUC1 staining. Pancreatobiliary type tumours are positive for MUC1 and negative for CDX2 and MUC2, irrespective of CK20 staining [1]. According to this triple system, tumours are classified as undetermined type in all immune profiles except these conditions. A 2-tiered approach has also been advocated based on which all tumours are pancreatobiliary histology if there is MUC1 positivity and CDX2 negativity, while the rest are considered as intestinal type [3, 4]. In cases where discordance was detected in all 3 evaluations, the CK7 and MUC5AC

IHC staining pattern, which was more frequently positive in pancreatobiliary subtypes, was used.

Immunohistochemical staining

Immunohistochemical staining was performed by preparing 3–4-μl thickness surface sections from formalin-fixed paraffin blocks using a Ventana BenchMark Ultra automatic IHC staining machine. Antibodies used for IHC staining were as follows: MUC1 (H23) PAb, MUC2 PAb, MUC5AC PAb, CDX2 (EPR2764Y) PAb, CK7 Rabbit Mono, CK20 Rabbit Mono, PD-1 (NAT105) PAb, and PD-L1 (SP263) PAb. Immunohistochemical examination was done by a pathologist experienced in pancreatobiliary cancers. In the IHC examination, for CK7, CK20, MUC1, MUC2, and MUC5AC cytoplasmic staining was accepted as positive, and for CDX2 nuclear staining was accepted as positive. PD1 was evaluated in immune cells and tumour-infiltrating lymphocytes (TIL), while PD-L1 was evaluated in immune cells, TIL, and tumour cells. For both antibodies, the percentage of viable target tumour cells with partial/complete staining at any intensity with respect to all viable tumour cells within the slide was determined. For PD1, more than 1% cytoplasmic/granular staining in immune cells and for PD-L1 more than 1% cytoplasmic/granular staining in tumour cells was considered positive.

Real-time polymerase chain reaction analysis

Formalin-fixed, paraffin-embedded resection specimens of ampullary adenocarcinoma containing more than 50% tumour cells were used. DNA was extracted from 5 paraffin sections (10 μm) representative of the tumour tissue by using a GeneJET Thermo Scientific extraction kit according to the manufacturer's protocol. The DNA concentration was measured by UV spectrometer and adjusted to 10 ~ 20 ng/μl. The extracted DNA was stored at –20°C until use. A PNAClamp™ EGFR Mutation Detection kit was used to detect EGFR mutations by RT-PCR. All reactions were performed in 20 μl volumes containing template DNA, primer and PNA probe sets, and fluorescence PCR master mix. All reagents were included in the kit. Real-time PCR reactions of PNA-mediated clamping PCR were performed using an Applied Biosystems™ 7500 Fast and 7500 RT-PCR. Polymerase chain reaction cycling conditions were a 5-min hold at 94°C, followed by 40 cycles of 94°C for 30 sec, 70°C for 20 sec, 63°C for 30 sec, and 72°C for 30 sec. Epidermal growth factor receptor mutation types were detected using PNA-mediated RT-PCR. The efficiency of PCR clamping was determined by measuring the threshold cycle (Ct) value. The target somatic mutations included E19 deletions, E21 L858R and L861Q mutation, E18 G719X mutation, E20 S768I mutation, E20 insertions, and E20 T790M mutation. Complete data analysis and quality control according to each department's specific protocols were performed.

Statistical analysis

SPSS 22.0 software was used in the analysis of the variables. The chi-square and Fisher's exact test were used for nonparametric variables, and Student's t-test was used for parametric variables in comparison of groups with normal distribution. The Mann-Whitney U test was used to compare the variables without normal distribution. In the survival analysis, the Kaplan-Meier test was used, and the effect of various variables on survival were evaluated with the log-rank test. Multivariate analysis of factors affecting survival was performed with Cox regression analysis. $P < 0.05$ was considered statistically significant. Overall survival (OS) was defined as the time from diagnosis to the last oncologic follow-up or death.

Ethics

The present study was supported by the Dokuz Eylul University Foundation of Scientific Research (2020.KB.SAG.047).

Results

Demographic and histopathological data

In our study, 83 ampullary adenocarcinoma patients who underwent pancreaticoduodenectomy were evaluated. The median age of the cases was 61 years (39–79); 48 (57.8%) were male and 35 (42.2%) were female. Nearly all patients had R0 resection ($n = 78$), and only 5 patients had positive surgical margins for malignancy (R1 resection). Sixty-eight patients had jaundice at the time of diagnosis while the other patients ($n = 15$) did not. Among all patients, baseline CEA and CA 19-9 data were available for 57 and 68 patients, respectively, with a median CEA level of 1.70 U/ml (1.2 ±11.9) and a CA 19-9 level of 94.31 U/ml (1.2 ±25232.4). Thirteen of the cases had grade 1 tumours, 53 cases had grade 2 tumours, and 17 cases had grade 3 tumours. In 48.2%, 49.4%, 60.2%, and 90.4% of the cases lymphovascular invasion, perineural invasion, pancreatic invasion, and duodenal invasion were noted, respectively.

When the pathological stages of the patients were evaluated, T3a (38.6%) tumours were the most common and T1a (2.4%) tumours were observed the least. According to the dissected lymph nodes of the cases, N0 disease was seen in 53.1% ($n = 44$) and lymph node metastasis was observed in 46.9% (N1: 36.1%, N2: 10.8%) of the patients. The tumour-node-metastasis stages at the time of diagnosis were 2 patients (2.4%) for Stage IA, 19 patients (22.9%) for Stage

IB, 17 patients (20.5%) for Stage IIA, 5 patients (6%) for Stage IIB, 28 patients (33.7%) for Stage IIIA, and 12 patients (14.5%) for Stage IIIB, respectively. Demographic and histopathological data of the patients are summarized in Table I.

Adjuvant and metastatic treatments

When the patients were analysed in terms of the adjuvant treatments they received, 5 patient groups were identified: adjuvant chemotherapy only ($n = 13$), adjuvant radiotherapy only ($n = 5$), adjuvant concomitant/sequential chemotherapy and radiotherapy ($n = 14$), adjuvant chemotherapy after chemoradiotherapy ($n = 19$), and no adjuvant treatment ($n = 32$). Forty-six patients (55%) received adjuvant chemotherapy alone or after RT or after chemoradiotherapy. The most common adjuvant regimen used was single-agent gemcitabine ($n = 22$), followed by gemcitabine + capecitabine or 5-fluorouracil (5-FU) ($n = 10$), gemcitabine plus cisplatin ($n = 4$), and single-agent capecitabine ($n = 1$). The median number of adjuvant chemotherapy cycles was 6 (range, 1–9). Chemotherapy regimens used in combination with radiotherapy were gemcitabine in 9 patients and capecitabine or 5-FU in 20 patients.

There were 42 patients (50.6%) who had local recurrence ($n = 10$) or metastases ($n = 32$) during or after adjuvant treatment. Of the 42 relapsed patients, 14 did not receive chemotherapy because of comorbidities, poor performance status, or patient rejection, and 27 patients received first-line chemotherapy (8 patients received single-agent gemcitabine, 8 patients received oxaliplatin + capecitabine or 5-FU, 3 patients received gemcitabine + capecitabine, 3 patients received gemcitabine + cisplatin, and 5 patients received other chemotherapy regimens). Eleven of 27 patients were able to receive second-line treatment, and only 7 of 11 patients were able to receive third-line treatment. In one patient complete response, in 4 patients partial response, and in one patient stable response was obtained with first-line treatments. Only stable response was observed in one patient with second-line treatments and in 2 patients with third-line treatments.

Immunohistochemical and polymerase chain reaction findings

CK7 staining was observed in 76 (91.6%) of the cases, and 100% staining was observed in 55 cases. Although staining with MUC5AC was detected in almost all cases ($n = 82$), 100% staining was observed in only 6 cases. In addition, the number of patients who stained positive with CK20, CDX2, MUC1, and MUC2 used in the CAP triple and dual assessment system was 48 (57.8%), 63 (75.9%), 81 (97.6), and 19 (22.9), respectively (Table II,

Table I. Patient characteristics*

Characteristics	Number of patients, n (%)
Sex	
Female	48 (57.8)
Male	35 (42.2)
Grade	
I	13 (15.7)
II	53 (63.9)
III	17 (20.5)
Stage	
IA	2 (2.4)
IB	19 (22.9)
IIA	17 (20.5)
IIB	5 (6.0)
IIIA	28 (33.7)
IIIB	12 (14.5)
Surgical margin	
R0	78 (94.0)
R1	5 (6.0)
Invasion type	
Lymphovascular invasion	40 (48.2)
Pancreatic invasion	50 (60.2)
Duodenal invasion	75 (90.4)
Perineural invasion	41 (49.4)
Jaundice at diagnosis	
Present	68 (18.1)
Absent	15 (81.9)

** Age: median – 61, range – 39–79 years*

Table II. Immunohistochemical staining results

Immunohistochemical markers	Number of patients with positive staining, n (%)
CK7	76 (91.6)
CK20	48 (57.8)
CDX2	63 (75.9)
MUC1	81 (97.6)
MUC2	19 (22.9)
MUC5AC	82 (98.7)
PD1	23 (27.7)
PDL1	19 (22.8)

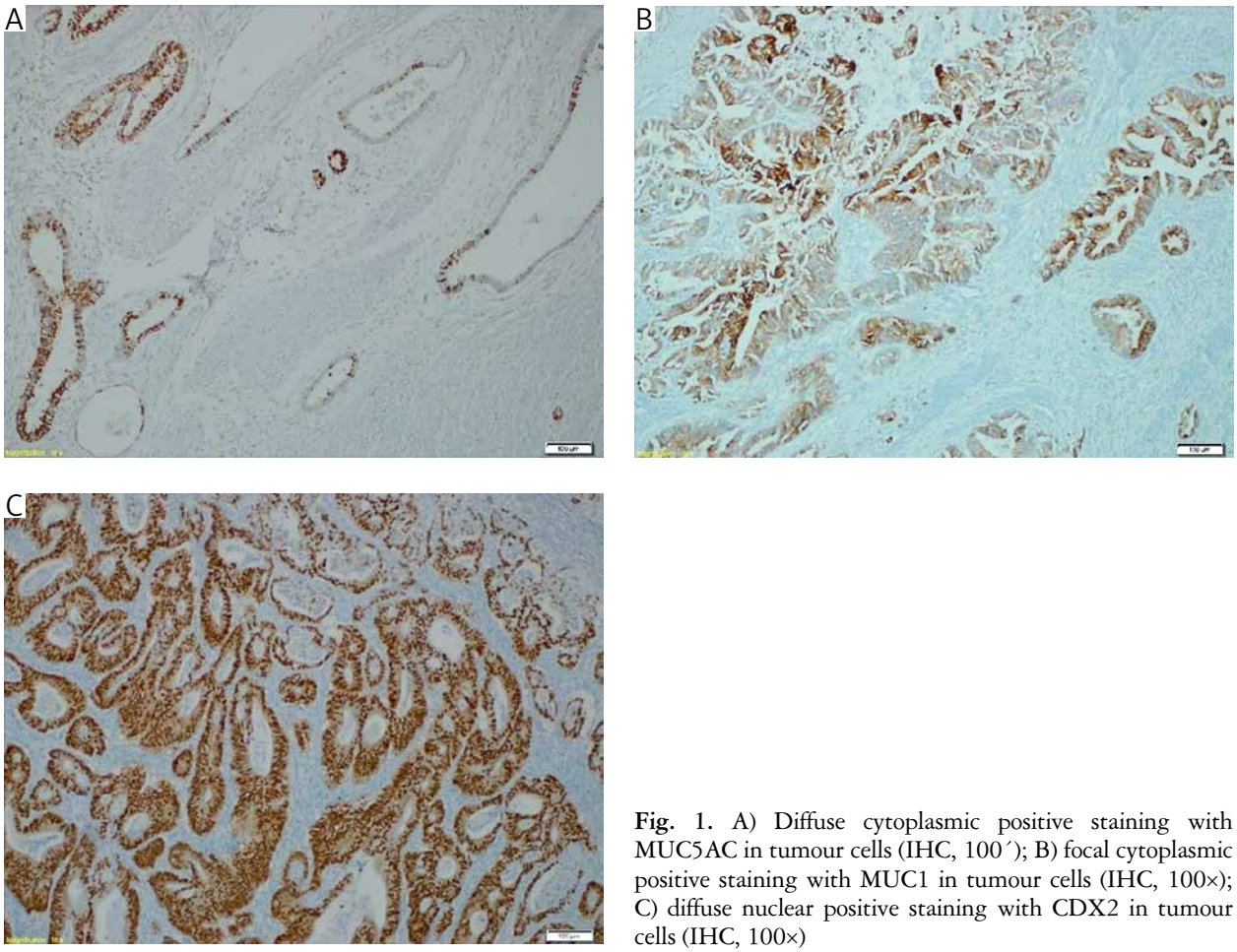

Fig. 1. A) Diffuse cytoplasmic positive staining with MUC5AC in tumour cells (IHC, 100´); B) focal cytoplasmic positive staining with MUC1 in tumour cells (IHC, 100×); C) diffuse nuclear positive staining with CDX2 in tumour cells (IHC, 100×)

Fig. 1). In the microscopic histopathological evaluation of adenocarcinoma subtypes with only haematoxylin-eosin stain, 42 (50.6%) were intestinal type, 29 (34.9%) were pancreatobiliary type, and 12 (14.5%) were found to be indeterminate. When histological subtypes were evaluated according to the recommendations of the CAP triple system, 17 (20.5%) were intestinal type, 22 (26.5%) were pancreatobiliary type, and 44 (53.0%) were undetermined. When histological subtypes were evaluated according to the recommendations of the CAP dual system, 62 (74.7%) were intestinal type and 21 (25.3%) were pancreatobiliary type. Based on these 3 different evaluation results, the subtypes of adenocarcinoma were finally defined. If the same subtype was detected in all 3 evaluations, a clear definition was made. If a discordance was found in one or 2 evaluations, the diagnosis was made by evaluating the existing findings and considering the CK7 and MUC5AC IHC staining patterns. Based on our final evaluation, 27 tumour tissues were determined to be pancreatobiliary type and 56 were intestinal type (Table III).

We compared the patients with respect to their PD-L1 and PD1 IHC staining; 19.6% (n = 11), 26.7% (n = 15) of intestinal type tumours and 25.9% (n = 7), 29.6% (n = 8) of pancreatobiliary type tumours were found to be positive, respectively (p = 0.260; p = 0.422) (Fig. 2). Epidermal growth factor receptor mutation was detected in a total of 6 patients (G719X mutation in 4 patients, L861Q mutation in one patient, and E19 deletion in one patient). Epidermal growth factor receptor mutation status could not be evaluated in 6 patients due to insufficient DNA isolation. Of these 6 mutations, 5 were observed in intestinal type tumours and one in a pancreatobiliary type tumour. However, the numerical difference did not reach statistical significance (p = 0.359).

Survival results

We analysed the OS time for all patients and found it to be 33 months (95% CI: 15.6–50.3). The median survival for patients who developed recurrence or metastasis was 15 months (95% CI: 11.37–18.62). The overall survival time of the patients with respect to stage was as follows: not reached for stage I (n = 21), 35 months for stage II (n = 22), and 19 months for stage III (n = 40) (p = 0.000). The median survival of patients with lymph node positive (n = 39) and negative (n = 44) was 20 months and 47 months, respectively

Table III. Ampullary cancer subtype assessment

Parameters	Number of patients, *n* (%)
Preliminary evaluation of histological subtype with haematoxylin-eosin staining	
Intestinal subtype	42 (50.6)
Pancreatobiliary subtype	29 (34.9)
Indefinite subtype	12 (14.5)
Histological subtype evaluation with immunohistochemistry results according to CAP triple system	
Intestinal subtype	17 (20.5)
Pancreatobiliary subtype	22 (26.5)
Indefinite subtype	44 (53.0)
Histological subtype evaluation with immunohistochemistry results according to CAP dual system	
Intestinal subtype	62 (74.7)
Pancreatobiliary subtype	21 (25.3)
Final evaluation	
Intestinal subtype	56 (67.5)
Pancreatobiliary subtype	27 (32.5)

CAP – College of American Pathologists

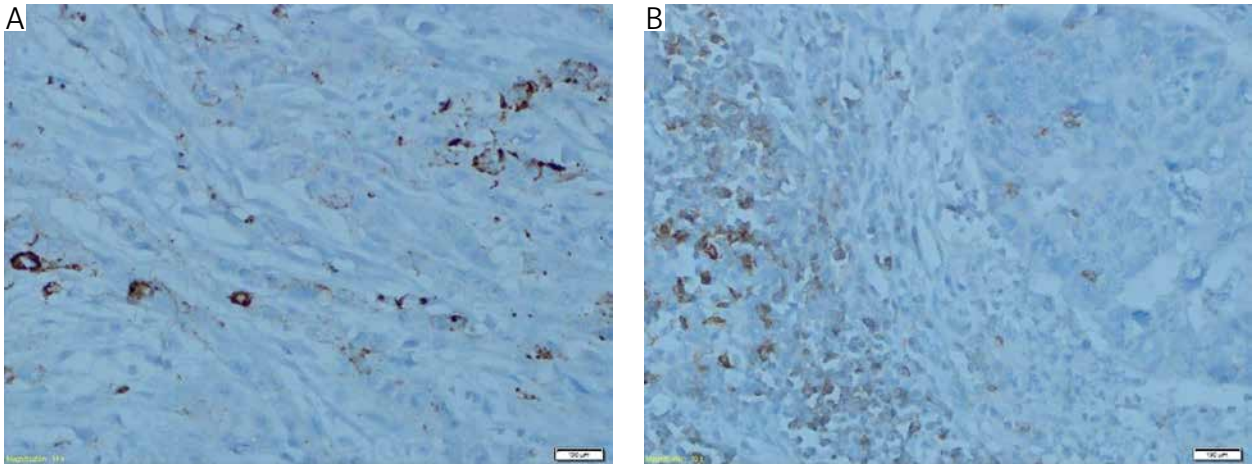

Fig. 2. A) Cytoplasmic/granular positive staining with PD-L1 in tumour cells (IHC, 200×); B) cytoplasmic/granular positive staining with PD-1 in tumour accompanying immune cells and tumour infiltrating lymphocytes (IHC, 200×)

($p = 0.001$) (Table IV). The median OS of patients with intestinal type adenocarcinoma ($n = 56$) was 23 months (95% CI: 9.38–36.61), while the median OS of patients with pancreatobiliary type adenocarcinoma ($n = 27$) was 76 months (95% CI: 26.91–125.09) ($p = 0.201$). When the median OS of PD1- and PD-L1-positive patients were compared with PD1- and PD-L1-negative patients, no significant difference was found ($p = 0.118$, $p = 0.884$). When the median OS of patients with EGFR mutations and those without EGFR mutations were compared, a significant difference was found ($p = 0.008$) (Fig. 3). Hence, the presence of EGFR mutation was found to have a negative and significant role in the prognosis of patients with ampullary cancer. In multivariate analysis, we identified T stage, N stage, and EGFR mutation status as independent prognostic factors for ampullary adenocarcinoma.

When we compared the median survival of patients who had no adjuvant treatment with the patients treated with any adjuvant therapy, we could not find any statistically significant difference (23 months; 95% CI: 0.0–70.6 vs. 33 months; 95% CI: 21.14–44.85; $p = 0.741$). The median survival values of all the treatment groups were each compared with the group of patients without any adjuvant treatment, and no statistical significance was found. When we analysed the adjuvant therapy in terms of intestinal and pancreatobiliary subtypes, we could not find any contribution

Table IV. Median survival in patient groups

Parameters	Number of patients, *n* (%)	Median survival (months)
Treatment		
Only adjuvant chemotherapy	13 (15.7)	35 ±3.37
Only adjuvant radiotherapy	5 (6.1)	36 ±24.1
Adjuvant CRT	14 (16.8)	28 ±12.1
Adjuvant chemotherapy after CRT	19 (22.9)	28 ±17.7
No adjuvant treatment	32 (38.5)	23 ±24.31
Grade		
I	21 (25.3)	77 ±11.52
II	22 (26.5)	35 ±23.26
III	40 (48.2)	19 ±1.89
Surgical margin		
R0	78 (93.9)	35 ±10.46
R1	5 (6.1)	13 ±15.14
Lymph node status		
Negative	44 (53.1)	47 ±9.0
Positive	39 (46.9)	20 ±3.11

CRT – chemoradiotherapy

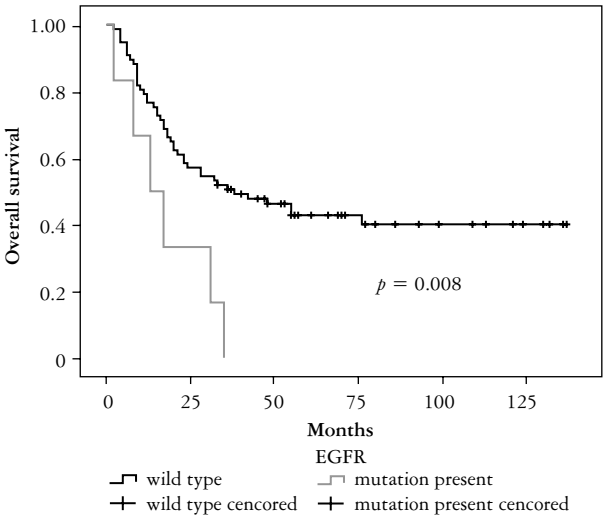

Fig. 3. Survival curve according to epidermal growth factor receptor mutation status

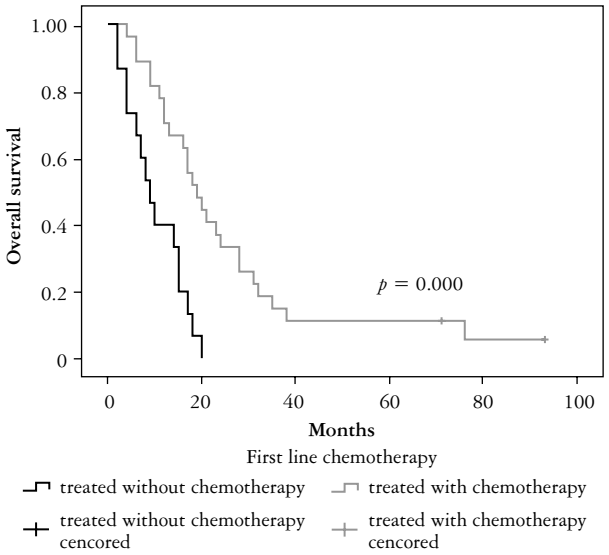

Fig. 4. Survival curve according to first-line chemotherapy

($p = 0.179$). However, when we compared the median survival of patients who received first-line chemotherapy ($n = 27$) with the patients who did not have first-line treatment ($n = 15$), a statistically significant difference was found (19 months; 95% CI: 13.91–24.08 vs. 9 months; 95% CI: 5.21–12.78; $p = 0.000$) (Fig. 4).

Discussion

Ampullary adenocarcinomas comprise 2 subtypes, which show many differences in histopathological features. The differentiation of these 2 subtypes is mostly possible with histopathological features in direct microscopic examination, but it is reported that this distinction can be made more easily and accurately by applying IHC markers. Although some criteria have been specified to distinguish these 2 subtypes according to their histopathological features and IHC staining pattern, different methodologies have been used in various studies. CD20, MUC1, MUC2, and CDX2 IHC staining patterns are recommended in the CAP ampullary carcinoma protocol. However, there may be patients who do not match the IHC staining pattern of the triple system, and this rate

was found to be as high as 44% in our study. On the other hand, IHC staining of MUC1 and CDX2 are used in the 2-tiered system, and by this method only a rough distinction can be made, which may be discordant with the microscopic examination results. Therefore, in our study, we evaluated all the existing markers that have been researched so far, especially in terms of IHC, and we think that we made a clear differentiation. We investigated both the IHC parameters used in the CAP classification protocol, and CK7 and MUC5AC, to better distinguish ampullary adenocarcinoma subtypes. Finally, tumours were divided into subtypes according to their histopathological features, 2-tiered, and triple classification protocol. If there was discordance in these classifications, then used CK7 and MUC5AC IHC staining patterns [15–17].

When the studies in the literature are examined, quite different results are found in terms of frequency of the ampullary adenocarcinoma subtypes. In a study conducted by Balcı *et al*. including 313 cases, it was reported that the frequency of pancreatobiliary type adenocarcinoma was 55%, intestinal type adenocarcinoma was 22%, and mixed and other tumours were 23% [18]. Similar to this study, there are many studies in the literature in which pancreatobiliary adenocarcinomas are more common than intestinal type adenocarcinomas. In these studies, the frequency of pancreatobiliary adenocarcinomas ranged from 44 to 72%, and it was stated that this subtype was predominant [19–22]. Conversely, both in the study done by Okano *et al*. and in a study from Memorial Sloan-Kettering Cancer Center, intestinal type adenocarcinomas were found to be predominant (60.6% and 49% of the cases were intestinal and 39.4% and 22% were pancreatobiliary, respectively) [23, 24]. In our study, 32.5% of the patients were pancreatobiliary type adenocarcinoma and 67.5% of the patients were intestinal type adenocarcinoma, and intestinal type was quite dominant compared to pancreatobiliary type.

It was thought that these 2 different subtypes might show different prognoses according to the tissue type to which these tumours are histologically similar. For example, most intestinal type adenocarcinomas are associated with adenoma, and although the data are conflicting, these tumours are reported to have a smaller diameter and better prognosis than others. In an Australian study that retrospectively evaluated 208 patients treated for ampullary adenocarcinoma, those with the histomolecular pancreatobiliary phenotype had a significantly worse prognosis than those with the intestinal phenotype [3]. On the other hand, other studies have concluded better survival outcome in favour of pancreatobiliary adenocarcinoma or no difference in OS between these subtypes of ampullary cancer [3–7]. In our study, although statistically non-significant, intestinal type adenocarcinoma was associated with inferior survival. In addition, we found tumour and lymph node status to be important determinants of prognosis. Therefore, while tumour and lymph node stage are important prognostic factors for ampullary cancer, the prognostic role of the histomolecular phenotype is not yet clear [25–28]. However, histomolecular phenotype may be more descriptive in terms of prognosis when evaluated together with lymph node status [3].

There are few published studies that can guide the use of adjuvant therapy in ampullary cancer patients, and the results of these clinical trials are uncertain. Most European clinicians consider adjuvant chemotherapy alone depending on the results of CONKO-001, EORTC, and ESPAC-1 studies [29–31]. The benefit of adjuvant chemotherapy for resected ampullary adenocarcinomas was directly examined in the ESPAC-3, and patients treated with gemcitabine achieved a median survival nearly twice as long as than those in the observation group [32]. The only randomized trial investigating the benefit of adjuvant chemotherapy is a multicentre randomized trial from Japan, which compared surgery alone to that with postoperative chemotherapy in 508 pancreatobiliary system cancer patients (ampullary cancer $n = 56$). A significant survival benefit was observed for adjuvant chemotherapy in patients with gallbladder cancer, but not in patients with ampullary cancer [33]. The American approach differs in terms of adjuvant therapy; for ampullary cancer patients with pathologic stage T2N0 or higher, it is recommended to add concomitant infusional 5-FU based chemoradiotherapy to adjuvant chemotherapy. The benefit of postoperative chemoradiotherapy in patients whose tumour was completely resected has been demonstrated in several uncontrolled series [34, 35]. Other retrospective studies and a single phase III randomized trial involving a significant number of patients with ampullary carcinoma failed to demonstrate any benefit for postoperative chemoradiotherapy [36–39]. We found that any kind of adjuvant treatment did not contribute to survival in our patients with ampullary cancer. Therefore, there is no consensus on the optimal management of ampullary cancer patients after complete tumour resection. It is also unclear whether these patients should be given adjuvant chemotherapy, adjuvant radiotherapy, or various combinations of these 2 treatment modalities.

Ampullary cancer can be detected earlier than other periampullary cancers because of the higher possibility of obstructive jaundice at early stages, and consequently the patients have a higher chance of curative surgical resection. For this reason, metastasis is not very common at the time of diagnosis in patients with ampullary cancer compared to those with tumours of hepatobiliary region. Studies on the treatment of advanced stage ampullary cancer

are either very few or are combined series that include patients with small intestine, pancreatic, and biliary tract cancers. Although gemcitabine-based combination chemotherapies have been recommended based on the ABC study, it is not yet clear whether to treat these patients like those with metastatic pancreatic cancer or small intestinal cancer [40, 41]. Thus, as for adjuvant treatment, there is no consensus on the best treatment approach for true ampullary cancer patients with metastatic disease. In our study, we showed that first-line chemotherapy contributed significantly to the survival of patients who developed relapse or metastases. If our patients did not receive gemcitabine in an adjuvant setting, they received gemcitabine ± cisplatin based treatments in the metastatic stage, but if they received gemcitabine in an adjuvant setting, they received 5-FU/capecitabine ± oxaliplatin based treatments in the metastatic stage.

PD1 and especially PD-L1 analysis in tumour tissue has gained importance due to the use of immunotherapies in the last decade. Many markers have been identified that can predict the effectiveness of immune checkpoint inhibitors in many solid tumours. Like tumour mutational burden and microsatellite instability, PD-L1 is also used as an important predictor to understand which patients will benefit from these treatments most. Immunotherapies may have a role in the treatment of advanced stage ampullary cancer patients with PD-L1 positivity when resistance develops to chemotherapies. Based on this, in our study we investigated both PD1 and PD-L1 IHC expression and found that nearly 20% of our patients had PD-L1-positive staining. When we searched the literature, we found 3 studies investigating PD-L1 in patients with ampullary cancer, and in the first one ($n = 76$), PD-L1 was positive in 90% of the patients, and high PD-L1 tumour expression was found to be associated with increased cancer-specific survival [42]. In the second and third studies, PD-L1 positivity was found in 26.9% ($n = 26$) and 44% ($n = 127$) of the patients, respectively [43, 44]. Discordant results could be due to the different antibody clones used in these studies. We could not reveal any prognostic significance of this biomarker in our study, but we believe that these molecules might be markers for predicting the responsiveness of immune checkpoint inhibitors in ampullary cancer.

Targeted therapies are preferred because they have fewer side effects and are more effective than chemotherapeutic agents. They have had many indications in patients with advanced stage cancer. For example, in lung cancer, inhibitors of EGFR, ALK, ROS1, MET, HER2, RET, NTRK1, MEK1, PIK3CA, and BRAF have replaced systemic chemotherapies in the treatment of tumours harbouring these molecular target mutations or rearrangements. In our study, we investigated the presence of EGFR mutation, which has many inhibitors that have been using in the treatment of solid tumours. We found EGFR mutations in approximately 7% of our patients. There are 2 studies each investigating EGFR expression and mutation [45–48]. Epidermal growth factor receptor mutation was found in 1% in one study and in 1 of 16 ampullary cancer patients in the second study [45, 46]. In a study investigating 93 pancreatic head and ampullary carcinoma patients, it was determined that survival outcomes of ampullary cancer patients are better than pancreatic cancer patients, which is possibly explained by differences in EGFR expression [47]. We also found the prognostic role of EGFR in ampullary carcinoma and concluded that the survival of patients with EGFR mutations was statistically significantly inferior to the patients without.

Conclusions

In our study, 28 patients died postoperatively, with a postoperative mortality rate of 22%, and the data of these patients were not included in analysis. In the literature, the postoperative mortality rate after the Whipple procedure was reported to be as high as 29%, while careful patient selection, improvements in surgical technique, and improvements in perioperative care have reduced this mortality rate to 2–4% in high-volume centres. In fact, pancreatobiliary cancers are advanced age diseases, and patients generally have many comorbidities, which increases both the morbidity and mortality of this complicated surgery. Another limitation of our study is the different adjuvant treatment categories (chemotherapy, radiotherapy, chemoradiotherapy, and post-chemoradiotherapy consolidation chemotherapy) and differences in chemotherapy regimens given in the metastatic period. However, the results are not surprising for a cancer that does not have such a treatment standard.

Ampullary carcinoma and its subtypes are difficult to evaluate due to their complex anatomical structure and hybrid histological features. We could not reveal any prognostic significance of PD1 and PD-L1, but we found that PD-L1 was positive in approximately one out of every 5 patients. We also investigated EGFR mutation and found that our patients with EGFR mutation had poorer overall survival. In conclusion, we have very limited knowledge about the treatment when recurrence or progression occurs in patients with ampullary cancer, which is a rare tumour that has a chance of cure with pancreaticoduodenectomy when detected early. So, considering that there are very few clinical studies in the literature, many large-scale and multi-centre studies are needed on these cancers.

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