Title: The clinicopathological differences of sporadic non-ampullary duodenal epithelial neoplasm depending on tumor location

Short title: Sporadic non-ampullary duodenal tumor

Abstract

Background and Aim: Although sporadic non-ampullary duodenal adenoma is speculated to be precancerous lesion, the relationship between adenoma and carcinoma remains unclear due to their rarity. Previous studies on sporadic non-ampullary duodenal epithelial neoplasm (SNADEN) have mainly targeted superficial tumors, like adenoma and early carcinoma. The clinicopathological features, including those of advanced carcinoma, remain poorly investigated. We assessed the clinicopathological features of SNADEN, including advanced carcinoma, focusing on tumor location.

Methods: We retrospectively collected the data of 410 patients who had been clinically and pathologically diagnosed with SNADEN at 11 institutions in Japan between June 2002 and March 2014.

Results: The SNADEN was mucosal neoplasia and invasive carcinoma in 321 (78.3%) and 89 (21.7) patients, respectively. The proportion of invasive carcinomas in SNADEN was significantly higher on the oral side of the papilla of Vater (oral-Vater) than on the anal side (anal-Vater) (27.9% vs. 14.4%, $P < 0.001$). Undifferentiated-type carcinoma was significantly more frequent with oral-Vater than anal-Vater (38.7% vs. 14.8%, $P = 0.026$). The recurrence rate of surgically R0 resected locally advanced carcinomas was significantly higher with oral-Vater than anal-Vater (46.4% vs. 8.3%, $P = 0.021$). Furthermore, the relapse-free survival with oral-Vater was significantly shorter than with anal-Vater (HR: 2.35; 95% confidence interval: 1.09–5.50; $P = 0.028$).

Conclusions: The clinicopathological features of SNADEN on oral-Vater were different from those on anal-Vater. SNADEN on oral-Vater was more likely to be invasive carcinomas and might behave more aggressively due to biologically higher

malignancy than that on anal-Vater.

Keywords Sporadic non-ampullary duodenal epithelial neoplasm (SNADEN); tumor location; clinicopathological differences

Introduction

Approximately 60% of duodenal adenomas develop in patients with familial adenomatous polyposis (FAP), while the remaining 40% are sporadic [1]. Sporadic non-ampullary duodenal adenoma (SNDA) is uncommon [2, 3], and little is known about its natural history. SNDA is thought to be a precancerous lesion, and the model of the adenoma-carcinoma sequence is predicted to involve the small intestine, including the duodenum and colorectum [4-6]. However, not all SNDA lead to carcinoma [7], and some previous reports have suggested that there was not only the adenoma-carcinoma sequence but also the *de novo* pathway in the carcinogenesis of duodenal carcinoma [7-10].

There have been several reports on the clinicopathological differences in superficial non-ampullary duodenal epithelial neoplasm according to the tumor location, particularly between the oral and anal side of the papilla of Vater [8, 9, 11]. Gastric-type tumors have been found to be more frequently located on the oral side of the papilla of Vater (oral-Vater) than on the anal side of the papilla of Vater (anal-Vater), and they are predicted to have a higher malignancy than intestinal-type tumors. In contrast, many of the tumors on anal-Vater are intestinal-type which are predicted to have lower malignant potential than gastric-type [8, 9]. However, since all of the previous studies examined data of only superficial tumors like adenoma and early carcinoma, the clinicopathological characteristics of sporadic non-ampullary duodenal epithelial neoplasm (SNADEN), including advanced carcinoma, are unclear.

As SNADEN is rare [12, 13], most previous studies were conducted at single tertiary-care centers. Furthermore, there has been no study including carcinoma in advanced stages. Data of these studies were potentially limited by selection bias and may not accurately reflect the true SNADEN status. In this study, we conducted a multicenter observational

study to elucidate the relationship between adenomas and carcinomas, including early and advanced stages, and the clinicopathological features of SNADEN according to the tumor location.

Methods

Patients

This was a multicenter, retrospective, observational study included a total of 410 patients at the following 11 hospitals from June 2002 and March 2014: Okayama University Hospital, Kurashiki Central Hospital, Okayama Saiseikai General Hospital, Hiroshima City Hiroshima Citizens Hospital, Japanese Red Cross Okayama Hospital, Kagawa Prefectural Hospital, Mitoyo General Hospital, Japanese Red Cross Society Himeji Hospital, Tsuyama Chuo Hospital, Sumitomo Besshi Hospital, and Akaiwa Medical Association Hospital. Patient data were collected after approval by the institutional review boards of each hospital. The ethics committee of each hospital approved this retrospective study and informed consent was acquired by the opt-out method.

Data collection

Data of patients with duodenal tumors with a histological diagnosis of adenoma or carcinoma were included in this study. The exclusion criteria were as follows: (1) tumor located on the ampulla of Vater, (2) familial adenomatous polyposis, (3) suspected invasive tumor of the pancreas, and (4) duodenal metastasis from the cancer of other organs. Patients' medical records were reviewed, and the following clinicopathological parameters were collected: gender, age, site of primary tumor, size of primary tumor,

morphology of primary tumor, histological type, symptoms at diagnosis, Union for International Cancer Control (8th ed.) cancer stage based on the tumor, nodes, metastasis (TNM) classification, treatment strategy, and patient prognosis. If a patient had multiple SNADENs, we evaluated the lesion that was the largest and most advanced. We investigated the clinicopathological features of SNADEN and compared the features according to the tumor location.

Histological examination

For histological analysis, tissue specimens were routinely fixed with formalin and completely embedded in paraffin. Tissue blocks were thinly sectioned, routinely processed, and stained with hematoxylin and eosin (H&E). All SNADENs were histologically graded based on the revised Vienna classification (VCL) [14]. Qualified pathologists at each hospital assessed histological grade. We defined VCL category 3 (low-grade dysplasia), 4.1 (high-grade dysplasia), 4.2 (carcinoma *in situ*), 4.3 (suspicious for invasive carcinoma), or 4.4 (intramucosal carcinoma) as mucosal neoplasia, and VCL category 5 (submucosal invasion by carinoma) as invasive carcinoma. We classified VCL category 3 as low-grade neoplasia, and 4 as high-grade neoplasia. Invasive carcinoma was subdivided into differentiated-type or undifferentiated-type depending on histopathological grading.

Statistical analysis

All continuous variables are reported as the median (range), and all categorical variables are summarized as frequencies (percentages). Wilcoxon’s rank-sum test was used to compare the continuous variables. Pearson's chi-square test or Fisher’s exact test

was used to compare the categorical variables. The overall survival (OS) and relapse-free survival (RFS) were estimated by the Kaplan–Meier method, and the differences were evaluated using the log-rank test. A Cox proportional hazard model was used to assess the OS and RFS by tumor location. All tests were two-sided, and a P-value under 0.05 was considered statistically significant. Statistical analyses were performed using the JMP 13 software program (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

Four-hundred and ten patients from the 11 institutions were included in this study, and their characteristics are summarized in Table 1. Median age of all patients was 67 years (range, 29–89), and 267 of the 410 (65.1%) patients were men while 143 (34.9%) were women. Of the 410 patients with SNADEN examined, 321 (78.3%) were diagnosed with mucosal neoplasia, and 89 (21.7%) were diagnosed with invasive carcinoma based on a histological examination of endoscopic biopsy or resected specimens. While the number of both mucosal neoplasias and invasive carcinomas were larger in men. Large tumor size and symptom were significantly correlated with invasive carcinoma ($P < 0.001$ and $P < 0.001$, respectively). Although advanced age was significantly correlated with invasive carcinoma, the difference was slight (median age, 67 vs. 68 years, $P = 0.0022$).

Comparison of the proportion of SNADEN between oral-Vater and anal-Vater

A total of 222 (54.1%) SNADENs were located on oral-Vater, while 188 (45.9%) were located on anal-Vater. Figure 1 shows the proportion of SNADEN according to the

tumor location. The proportion of invasive carcinomas on oral-Vater was significantly higher than anal-Vater (27.9% vs. 14.4%, $P < 0.001$). Similarly, the proportion of both stage I invasive carcinomas and stage II-IV invasive carcinomas were significantly higher on oral-Vater than anal-Vater (3.6% vs. 0.5%, $P = 0.034$, and 24.3% vs. 13.8%, $P = 0.0075$, respectively).

Comparison between oral-Vater and anal-Vater in mucosal neoplasias and invasive carcinomas

The 321 mucosal neoplasias were divided into 160 lesions on oral-Vater and 161 lesions on anal-Vater (Table 2). There were no significant differences in sex, age, or tumor size between the two groups. Regarding histological type, the proportion of high-grade neoplasia was significantly higher on oral-Vater than on anal-Vater (28.7% vs. 19.3%, $P = 0.046$). Protruded-type was significantly more frequent on oral-Vater, while superficial-type was significantly more frequent on anal-Vater ($P < 0.001$).

The 89 invasive carcinomas were divided into 62 lesions on oral-Vater and 27 on anal-Vater. The results of univariate analysis between the two groups among invasive carcinomas are summarized in Table 3. There were no significant differences in sex, age, tumor size, symptom, or TNM stage between the two groups. With regard to histological type, the proportion of undifferentiated-type carcinomas was significantly higher on oral-Vater than on anal-Vater (38.7% vs. 14.8%, $P = 0.026$). The 28 undifferentiated-type carcinomas were all advanced carcinomas.

The prognosis of advanced carcinomas according to tumor location

Regarding stage II-IV invasive carcinomas, 55 lesions were located on oral-Vater,

while 24 were located on anal-Vater. There was no significant difference in stage or OS between the two groups (median OS: 16.0 vs. 20.6 months, $P = 0.88$).

We defined carcinomas invading the muscularis propria (T2) and deeper (T3 and T4) without distant metastasis as locally advanced carcinomas. The 40 patients with locally advanced carcinomas received surgical resection with no residual tumor (R0). The surgically R0 resected locally advanced carcinomas were divided into 28 lesions on oral-Vater (oral-Vater group) and 12 on anal-Vater (anal-Vater group). Table 4 shows a comparison of the characteristics of patients with surgically R0 resected locally advanced carcinomas according to the tumor location. There was no significant difference in stage between the two groups. Postoperative recurrence occurred significantly more often on oral-Vater than on anal-Vater (46.4% vs. 8.3%, $P = 0.021$). Kaplan–Meier curves of RFS by tumor location are shown in Figure 2. The median RFS for the oral-Vater and anal-Vater groups was 17.1 and 63.6 months, respectively. The RFS of the oral-Vater group was significantly shorter than that of the anal-Vater group (HR: 2.35; 95% confidence interval: 1.09–5.50; $P = 0.028$). Similarly, the median OS of 19.6 months in the oral-Vater group was shorter than that of 63.6 months in the anal-Vater group, but the difference was not statistically significant ($P = 0.11$).

Discussion

This study is the first to focus on the relationship between mucosal neoplasias and invasive carcinomas, including early and advanced stages, and the clinicopathological features according to the tumor location, as described in the introduction. From this study, the incidence of invasive carcinomas, including early and advanced stage, was found to be correlated with the tumor location, and the clinicopathological features of

advanced carcinoma were found to differ between oral-Vater and anal-Vater. The incidence of invasive carcinomas on oral-Vater was higher, and the clinical behavior of advanced carcinomas on oral-Vater was worse, compared with that on anal-Vater.

The clinicopathological features of SNADEN were different depending on the tumor location in the current study. The proportion of invasive carcinomas on oral-Vater was significantly higher than on anal-Vater, regardless of the stage. As a result, it turned out that SNADEN on oral-Vater seemed to have worse malignant potential than that on anal-Vater. The proportion of both high-grade neoplasias and undifferentiated-type carcinomas were also significantly higher on oral-Vater than on anal-Vater. It is known that the mucosa in the proximal duodenum can potentially undergo gastric metaplasia in the duodenum (GMD) caused by exposure to gastric acid [15]. The GMD is thought to be a potentially precancerous state, leading to *de novo* carcinoma on oral-Vater [16]. This may be one of the reasons for which oral-Vater SNADEN tends to be diagnosed as invasive carcinoma and undifferentiated-type carcinoma.

We investigated the prognosis and postoperative recurrence in order to verify the difference in the grade of malignancy according to the tumor location. Regarding stage II-IV invasive carcinomas, there were no significant differences in OS between oral-Vater and anal-Vater. In contrast, regarding surgically R0 resected locally advanced carcinomas, postoperative recurrence occurred significantly more often on oral-Vater than on anal-Vater. Furthermore, the median RFS of 17.1 months in the oral-Vater group was significantly shorter than that of 63.6 months in the anal-Vater group. The matters of undifferentiated-type mentioned above, postoperative recurrence, and RFS suggested that advanced carcinomas on oral-Vater aggressively behave as biologically higher malignancy. Previous studies have reported that there were the

histopathological differences of superficial non-ampullary duodenal epithelial neoplasm between oral-Vater and anal-Vater [8, 9, 11]. Our date clarified the differences in clinical behavior of invasive carcinomas, including early and advanced stages, according to the tumor location.

This study has several limitations. First, this was a retrospective study. Future prospective research should be conducted to further clarify the association between malignancy of SNADEN and tumor location. However, our clinical data revealed differences in the clinicopathological features of SNADEN, including advanced carcinoma, between oral-Vater and anal-Vater. Therefore, the results of this study might help future research related to the pathogenesis of SNADEN according to the tumor location. Second, because some patients with locally advanced carcinomas underwent partial resection without lymphadenectomy, we excluded these cases from the analysis of surgically R0 resected locally advanced carcinoma. This might have resulted in some degree of selection bias in this analysis. However, the cases of partial resection without lymphadenectomy in both groups was small and almost same, and we believe that the substantial difference in the median RFS was extremely meaningful.

In conclusion, the clinicopathological features of SNADEN, including advanced carcinoma, differed between oral-Vater and anal-Vater as well as superficial non-ampullary duodenal epithelial neoplasm. SNADEN on oral-Vater might have significantly higher malignant potential than on anal-Vater.

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