Original article

Measurement of Serum Marker for Bone Metastasis (1-CTP) in Hepatobiliary and Pancreas Malignancies

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ABSTRACT

Background/Aims: Pyridinoline cross-linked carboxyterminal telopeptide of type Ⅰ collagen（1CTP）is a sensitive serum marker for metastatic bone carcinomas and may also be associated with invasiveness of various carcinomas. To clarify the significance of 1CTP in hepato-biliary pancreas malignancies, we examined the relationship between clinicopathological features and serum level of 1CTP.

Methodology: The subjects were 75 patients who underwent surgical resections including 27 patients with liver carcinomas, 15 with extra-hepatic biliary carcinomas, 14 pancreatic carcinomas and 19 benign diseases.

Results: 1 CTP level tended to be higher in the malignant diseases than in benign diseases but this difference was not significant (p=0.065). Compared to benign adenoma, 1CTP level in the malignant diseases was significantly higher (p=0.049). 1CTP level tended to be higher in patients with cholangitis compared to those with no inflammation or benign tumors (p=0.065). 1CTP was not correlated with any tumor markers. 1CTP was not associated with node status and vascular infiltrations. 1CTP level tended to be lower in patients with poor differentiation.

Conclusions: Serum level of 1CTP might be a predictive marker for hepatobiliary pancreas malignancies but also reflects the degree of co-existing cholangitis.

Key Words: hepatobiliary pancreas; 1CTP; cholangitis

Abbreviations: Pyridinoline cross-linked carboxyterminal telopeptide of type Ⅰ collagen（1CTP）;

INTRODUCTION

In hepatic, biliary and pancreatic malignant diseases, some serum tumor markers have been used for diagnosis (1-3); however, markers to predict invasiveness or metastasis in these diseases have not been fully clarified yet. Pyridinoline cross-linked carboxyterminal telopeptide of type Ⅰ collagen (1CTP) is a 12,000 molecular weight uniform peptide in the serum and is produced by bone absorption or destruction (4). This serum peptide is a sensitive marker for bone metastasis from adenocarcinomas, bone malignancies, bone fracture, systemic inflammatory diseases, hepatic fibrosis etc (5-8). In some malignant diseases, 1CTP increases in patients with pancreatic carcinomas (9). Our preliminary study of 1CTP in colorectal carcinomas showed that a higher level of this marker seemed to be associated with depth of invasion (not published yet). We hypothesize that this marker is associated with tumor invasiveness in adenocarcinomas, and can be used as a predictive marker for invasiveness in hepatobiliary pancreas malignancies.

In the present study, we examined the serum level of 1CTP in benign and malignant diseases in the liver, extrahepatic bile duct and pancreas and the relationship between 1CTP level and clinicopathological features to investigate clinical significance.

METHODOLOGY

The subjects were 75 patients who underwent surgical resections including 37 patients with liver carcinomas, 15 with extra-hepatic biliary carcinomas, 13 with pancreatic carcinomas and 10 with benign diseases, in the Division of Surgical Oncology, Department of Translational Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences (NUGSBS) between 2007 and February 2009. They included 53 males and 22 females with a mean age of 65.7±12.5 years (±SD, range, 36-86 years).

Liver diseases included hepatocellular carcinoma (n=21), intrahepatic cholangiocarcinoma (n=6), metastatic liver carcinoma (n=10), liver cyst (n=2), hemangioma (n=1) and hepatolithiasis (n=2). The extrahepatic biliary diseases included gallbladder carcinoma (n=6), bile duct carcinoma (n=9), adenoma of gallbladder (n=1) and cholecystolithiasis (n=1). Pancreatic diseases included pancreatic carcinomas (n=13), intraductal papillary mucinous adenoma (n=1) and pancreatitis (n=2). The background liver diseases included chronic viral liver diseases (n=25; caused by viral hepatitis [n=21] or alcoholic [n=4]), cholangitis (n=16) and pancreatitis (n=12). No patients had clinical bone metastasis during the examinations.

The study design was approved by the Ethics Review Board of our institution and a signed consent for a study using blood sample or laboratory data was obtained from each patient before operation. The present analysis was a retrospective study. Data were retrieved from patient charts in the NUGSBS database.

The blood samples were preoperatively collected in the morning before meal and the serum was stored at -20℃. The serum samples were used for measurement of 1CTP

by the radioimmunoassay-2 antibody method kit (BML Inc., Tokyo) and data was collected every couple of days. The normal range of concentration of 1CTP is less than 4.5 ng/ml by the company record.

All continuous data were expressed as mean ± SD. Data for different groups were compared using one-way analysis of variance (ANOVA). Chi-square test was used for comparison of categorical variables. Differences between groups were analyzed by Fisher's exact test or Scheffé's multiple comparison test. A two-tailed P value of less than 0.05 was considered significant. StatView Software for Windows, version 5.0 (SAS Institute, Inc., Cary, NC) was used in all statistical analyses.

RESULTS

Table 1 shows the concentration of 1CTP between benign and malignant disease. Serum 1 CTP level tended to be higher in the malignant diseases than in benign diseases but not significantly. Compared to benign tumors such as adenoma of gall bladder and intraductal papillary mucinous adenoma, 1CTP level in the malignant diseases was significantly higher. Incidence of high 1CTP level (≥4.5ng/ml) tended to also be higher in the malignant diseases than in benign diseases, but this difference was not significant. In malignant diseases, 1CTP level or incidence of higher 1CTP were not significantly different between diseases. By focusing on the inflammation of background organs, 1CTP level tended to be higher in patients with cholangitis compared to those with no inflammation or benign tumors although there were no statistical differences.

Table 2 shows the correlation between 1CTP and other serum tumor markers in hepatobiliary pancreas malignancies. 1CTP was not correlated with any tumor markers.

Table 3 shows the relationship with histological findings. 1CTP was not associated with node status and vascular infiltrations. 1CTP level tended to be lower in patients with poor differentiation and there were no cases with higher 1CTP level≥4.5ng/ml.

DISCUSSION

1CTP was reported as a useful bone resorption marker in 1993 and this marker was analyzed for bone metabolism in hormonal diseases, osteoporosis, and bone metastasis from prostate, lung, breast and pancreatic carcinomas (5-13). During rule-out bone metastasis in patients with colorectal carcinomas at our institutes, 1CTP was increased slightly more than normal range (≥4.5ng/ml) in some patients without clinical bone metastasis or other bone diseases (reported at a Japanese congress but not published yet). In these cases, tumor invasion was deeper than that expected before operation by endoscopic ultrasonic diagnosis. Based on our experience, we hypothesized that this marker could be detectable in tumor invasion or surrounding metastasis in digestive tract carcinomas at an earlier stage. In carcinomas of the liver, biliary and pancreas, precise preoperative diagnosis of tumor invasion cannot be fully obtained because of anatomical limitations. Particularly, the depth of invasion to serosa or node metastasis is an extremely important factor to predict patient outcomes, and selection of operative procedure was often different according to intraoperative findings of tumor invasiveness (14, 15). To expect tumor invasiveness or aggressiveness preoperatively, we considered that 1CTP was a candidate marker in the present study. A previous report showed the usefulness of bone metastasis in pancreatic carcinomas (9); however, this is the first trial with the goal to determine tumor invasiveness in hepatobiliary pancreas carcinoma, to our knowledge.

Our results showed a slight difference of 1CTP level between benign and malignant diseases in general, although there were no significant differences. As no remarkable bone metastases have been found in our series, 1CTP might be sensitive for cancer

diagnosis. In our series, we examined pre-cancerous lesions (=adenomas) and serum 1CTP level was significantly lower than in cancer lesions. Therefore, it may be possible to use 1CTP level for a differential diagnosis between pre-cancerous and cancerous diseases. As shown in the results, 1CTP was not correlated with any existing tumor markers and, therefore, it could be used as an independent sensitive marker to diagnose malignancy in hepatobiliary pancreas lesions. In these lesions, characteristics or malignant behaviors are quite different between diseases, and 1CTP level was higher than in benign diseases. 1CTP level in biliary carcinomas seemed to be higher than those in liver and pancreas carcinomas but the difference was not significant. Therefore, further examination in a larger series will be necessary to clarify 1CTP levels in each disease.

On the other hand, serum 1CTP level was different with the existence of background inflammation in our results. Compared to patients without inflammation, the co-existence of inflammatory disease tended to be associated with an increase of 1CTP level, particularly cholangitis. Previous reports showed that 1CTP level was increased in inflammatory diseases such as hepatic fibrosis or rheumatoid arthritis (5, 16). In cases with biliary diseases, modification to 1CTP level by cholangitis should be considered and it is necessary to take measurements once inflammation has subsided.

Although our first aim in examining 1CTP was to evaluate tumor invasiveness, 1CTP level was not correlated with vascular infiltration or node metastasis in the present results. In each carcinoma, 1CTP level was not correlated with tumor stage or depth of invasion (data not shown due to limitation of number), either. Therefore, our hypothesis was not clarified in the present study. Interestingly, 1CTP level was much lower in

poorly differentiated carcinomas. 1CTP might be produced by highly differentiated cancer cells, although no reports showed this result yet.

In conclusion, we have examined serum 1CTP level as a marker of tumor invasiveness in hepatobiliary pancreatic diseases. Serum 1CTP level tended to be higher in malignant diseases than in benign diseases and this level was not associated with type of cancer or tumor invasiveness. Serum 1CTP level was not higher in poorly differentiated carcinomas. 1CTP level was also increased in patients with coexisting cholangitis. Our results highlight the clinical usefulness of measuring 1CTP for patients with hepatobiliary pancreas malignant diseases and future studies in a larger number of patients is necessary to clarify usefulness.

REFERENCES

1. **Pang RW, Joh JW, Johnson PJ, Monden M, Pawlik TM, Poon RT:** Biology of hepatocellular carcinoma. Ann Surg Oncol. 2008;15:962-971.
2. **Ni XG, Bai XF, Mao YL, Shao YF, Wu JX, Shan Y, Wang CF, Wang J, Tian YT, Liu Q, Xu DK, Zhao P:** The clinical value of serum CEA, CA19-9, and CA242 in the diagnosis and prognosis of pancreatic cancer. Eur J Surg Oncol. 2005;31:164-169.
3. **Ghosh M, Koike N, Tsunoda S, Hirano T, Kaul S, Kashiwagi H, Kawamoto T, Ohkohchi N, Saijo K, Ohno T, Miwa M, Todoroki T:** Characterization and genetic analysis in the newly established human bile duct cancer cell lines. Int J Oncol. 2005;26:449-456.
4. **Miyakawa M, Tsushima T, Demura H:** Carboxy-terminal propeptide of type 1 procollagen (P1CP) and carboxy-terminal telopeptide of type 1 collagen (1CTP) as sensitive markers of bone metabolism in thyroid disease. Endocr J. 1996;43:701-708.
5. **Engvall IL, Svensson B, Tengstrand B, Brismar K, Hafström I; the BARFOT study group:** Impact of low-dose prednisolone on bone synthesis and resorption in early rheumatoid arthritis: experiences from a two-year randomized study. Arthritis Res Ther. 2008;10:R128.
6. **Bath LF, Crofton PM, Evans AE, Ranke MB, Elmlinger MW, Kelnar CJ, Wallace WH:** Bone Turnover and Growth during and after Chemotherapy in Children with Solid Tumors. Pediatr Res. 2004;55:224-230.
7. **Kiuchi K, Ishikawa T, Hamaguchi Y, Momiyama N, Hasegawa S, Ishiyama A, Kono T, Doi T, Chishima T, Shimada H:** Cross-linked collagen C- and N-telopeptides for an early diagnosis of bone metastasis from breast cancer. Oncol Rep. 2002;9:595-598

8. **Miyakawa M, Tsushima T, Demura H:** Carboxy-terminal propeptide of type 1 procollagen (P1CP) and carboxy-terminal telopeptide of type 1 collagen (1CTP) as sensitive markers of bone metabolism in thyroid disease. Endocr J. 1996 ;43:701-708.
9. **Iguchi H, Yasuda M, Matsuo T, Sumii T, Funakoshi A:** Clinical features and management of pancreatic cancer with bone metastases. (in Japanese with English abstract) Nippon Shokakibyo Gakkai Zasshi. 2004;101:872-878.
10. **Izumi M, Nakanishi Y, Takayama K, Kimotsuki K, Inoue K, Wataya H, Minami T, Hara N:** Diagnostic value of bone-turnover metabolites in the diagnosis of bone metastases in patients with lung carcinoma. Cancer. 2001;91:1487-1493.
11. **Okazaki Y, Tsurukami H, Nishida S, Okimoto N, Aota S, Takeda S, Nakamura T:** Prednisolone prevents decreases in trabecular bone mass and strength by reducing bone resorption and bone formation defect in adjuvant-induced arthritic rats. Bone. 1998;23:353-360.
12. **Abildgaard N, Glerup H, Rungby J, Bendix-Hansen K, Kassem M, Brixen K, Heickendorff L, Nielsen JL, Eriksen EF:** Eur J Haematol. Biochemical markers of bone metabolism reflect osteoclastic and osteoblastic activity in multiple myeloma. 2000;64:121-129.
13. **Peris P, Alvarez L, Monegal A, Guañabens N, Durán M, Pons F, Martínez de Osaba MJ, Echevarría M, Ballesta AM, Muñoz-Gómez J:** Biochemical markers of bone turnover after surgical menopause and hormone replacement therapy. Bone. 1999;25:349-353.
14. **Nagino M, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y:** Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. Ann Surg. 2006;243:364-372.

15. **Kai M, Chijiiwa K, Ohuchida J, Nagano M, Hiyoshi M, Kondo K:** A curative resection improves the postoperative survival rate even in patients with advanced gallbladder carcinoma. J Gastrointest Surg. 2007;11:1025-1032.
16. **Stone PJ:** Potential use of collagen and elastin degradation markers for monitoring liver fibrosis in schistosomiasis. Acta Trop. 2000;77:97-99.

TABLE 1. Comparison of 1CTP Concentrations in Benign and Malignant Diseases in Hepatobiliary-pancreas.

	1CTP concentration	**Ratio of higher level (≥4.5ng/ml)**
Benign diseases	5.3 ± 4.3	3/10 (30)
Benign tumors	3.7 ± 1.8	1/3 (33)
Malignant diseases	6.4 ± 4.3*†	37/ 65 (58)
1) Liver diseases	5.6 ± 2.5	22/37 (60)
Hepatocellular carcinoma	5.8 ± 2.5	12/21(57)
Intrahepatic cholangiocarcinoma	6.7 ± 3.6	4/ 6 (67)
Metastatic liver carcinoma	5.2 ± 1.3	6/10(60)
2) Biliary diseases	8.2 ± 6.1	9/15 (60)
Gall bladder carcinoma	7.2 ± 4.5	3/ 6 (50)
Bile duct carcinoma	8.9 ± 7.2	6/ 9 (67)
3) Pancreatic carcinomas	6.2 ± 5.4	6/13 (49)
Background inflammation		
No inflammation	5.1 ±2.4	12/28 (43)
Hepatitis	5.7± 2.5	15/25 (60)
Cholangitis	7.8 ± 6.0 #	8/16 (50)
Pancreatitis	7.7 ± 6.2	8/12 (67)

*p=0.065 vs. benign diseases. †p=0.049 vs. benign tumors.

#p=0.065 vs. no inflammation group, p=0.05 vs. benign tumors.

Data are mean±SD

TABLE 2. Correlations Between 1CTP Concentrations and Other Tumor Markers.

	Correlation coefficient (r)	**p value**
Carcinoembryonic antigen	-0.02	0.84
CA19-9	0.01	0.94
Alpha-feto protein (AFP)	-0.11	0.50
AFP L3 subclass	0.12	0.59
Des-γ-carboxy prothrombin	-0.09	0.66
Elastase 1	-0.12	0.55
DUPAN-II	-0.15	0.68

TABLE 3. Relationship Between 1CTP Concentrations and Pathological Findings.

	1CTP concentration	**Ratio of higher level (≥4.5ng/ml)**
Lymph node metastasis		
No	6.1 ± 3.2	13/22 (59)
Yes	5.8 ± 2.8	7/14 (50)
Vascular involvement		
No	5.9 ± 2.8	20/31(65)
Yes	5.2 ± 2.5	8/20 (40)
Histological differentiation		
Well	6.9 ± 3.5	9/11 (88)
Moderately	5.3 ± 2.2	14/24 (58)
Poorly	4.0 ± 0.3†	0/ 8 (0)*

†p=0.10 and *p<0.01vs. well differentiated carcinomas