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Original article

Specific increase in serum core-fucosylated haptoglobin in patients with chronic pancreatitis

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ABSTRACT

Background/Objectives: Pancreatic ductal adenocarcinoma (PDAC) has the worst prognosis of all malignancies, and its diagnosis in early stages is the most important prognostic factor. Chronic pancreatitis (CP), a common background of PDAC occurrence, is morphologically defined as progressive pancreatic fibrosis and inflammation accompanied by pancreatic exocrine cell atrophy. We recently found that inflammation and fibrosis are independent characteristic histological changes in noncancerous lesions in PDAC patients despite the absence of a past history of clinical CP. Subclinical CP is an important background for PDAC occurrence. Therefore, there is an urgent need to develop a noninvasive and reliable biomarker for CP diagnosis.
Methods: Fifty-nine healthy volunteers (HV), 159 patients with CP, and 83 patients with PDAC were enrolled in this study. We measured serum total fucosylated haptoglobin (Fuc-Hpt) and core-Fuc-Hpt levels using lectin-antibody enzyme-linked immunosorbent assay kits that we developed. In these kits, total Fuc-Hpt and core-Fuc-Hpt were measured using *Aleuria aurantia* lectin and *Pholiota squarrosa* lectin, respectively.
Results: Serum Fuc-Hpt levels were significantly increased in CP patients compared to HV ($P < 0.0001$) and were further increased in PDAC patients ($P < 0.0001$). Interestingly, serum core-Fuc-Hpt levels were significantly higher in CP patients compared to HV ($P < 0.0001$) and PDAC patients ($P < 0.0001$). Multivariate analyses demonstrated that total serum core-Fuc-Hpt was an independent determinant for CP diagnosis, but Fuc-Hpt was not.
Conclusions: A dramatic change in oligosaccharides was observed in serum haptoglobin between CP and PDAC. Serum core-Fuc-Hpt may be a novel and useful biomarker for CP diagnosis.

Introduction

Chronic pancreatitis (CP) is characterized by progressive destruction of the pancreas tissue and recurrent episodes of intractable abdominal pain accompanied by exocrine and endocrine pancreatic insufficiencies [1,2]. CP is a strong risk factor for pancreatic ductal adenocarcinoma (PDAC) occurrence [3–5]. Interestingly, surgical intervention of CP reduces PDAC occurrence [6]. Morphologically, CP is defined as progressive pancreatic fibrosis

Abbreviations: AAL, *Aleuria aurantia* lectin; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; AST, aspartate aminotransferase; AUROC, area under the ROC curve; CEA, carcinoembryonic antigen; CP, chronic pancreatitis; Cr, creatinine; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; Fuc-Hpt, fucosylated haptoglobin; GGT, γ-glutamyltransferase; HV, healthy volunteer; PDAC, pancreatic ductal adenocarcinoma; PhoSL, *Pholiota squarrosa* lectin; ROC, receiver operating characteristic; T-Bil, total bilirubin; T-Chol, total cholesterol; TG, triglyceride.

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and inflammation that is accompanied by atrophy of pancreatic parenchymal cells [1,2].

Recently, we investigated 76 PDAC patients who underwent surgery and analyzed pancreatic histological changes in specimens of noncancerous lesions [7]. We found that pancreatic inflammation and fibrosis were significant and independent determinants for PDAC. These histological changes were exactly the same characteristic histological changes observed in CP. Surprisingly, none of the 76 PDAC patients had a past history of clinical CP. Considering these findings, subclinical CP is an important risk factor for PDAC occurrence. However, no useful clinical biomarkers for CP have been identified.

Fucosylation is one of the most important types of glycosylation involved in cancer and inflammatory diseases [8]. Several types of fucosylated glycoproteins have been identified as cancer biomarkers including fucosylated alpha-fetoprotein, referred to as AFP-L3 [9]. Based on results from lectin blot analysis, we previously reported that fucosylated haptoglobin (Fuc-Hpt) is a novel biomarker for pancreatic cancer [10]. Although an increase in the level of Fuc-Hpt has been observed in other cancers as well as in benign inflammatory diseases, the positive rate of Fuc-Hpt is the highest level in pancreatic cancer. To measure the serum levels of Fuc-Hpt, we developed a lectin-antibody enzyme-linked immunosorbent assay (ELISA) [11,12]. In this lectin-antibody ELISA system, we used *Aleuria aurantia* lectin (AAL), which recognizes all types of fucosylation. In our previous study, we demonstrated that the Lewis type of fucosylation (α1–3/α1–4 fucosylation) exhibits the greatest increase and that core-fucosylation (α1–6 fucosylation) is increased slightly in the sera of PDAC patients [13]. Core-fucosylation of glycoproteins is widely distributed and is altered under pathological conditions [14]. For example, the absence of immunoglobulin G core-fucose greatly enhances antibody-dependent cellular cytotoxicity [15] and changes growth factor signaling [16,17].

Previously, we identified a novel lectin, *Pholiota squarrosa* lectin (PhoSL), which specifically recognizes core-fucosylation [18]. Using PhoSL, we recently developed a novel lectin-antibody ELISA system that can measure serum core-fucosylated haptoglobin in humans [22]. In the present study, we measured serum total Fuc-Hpt (AAL-Hpt) and core-Fuc-Hpt (PhoSL-Hpt) using the lectin-antibody ELISA systems we developed in 59 healthy volunteers (HV), 159 patients with CP, and 83 patients with PDAC. Interestingly, we found that core-Fuc-Hpt is a novel serum biomarker for distinguishing patients with CP from HV and patients with PDAC. Our findings should enable the noninvasive screening of subclinical CP in the clinic.

Materials and methods

Ethics committee approval

This study was approved by the ethics committee of Osaka University Hospital (No. 260), and the study was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all subjects at the time of enrollment or blood sampling.

Study subjects

Fifty-nine HV subjects were enrolled in this study from the aMs New Otani Clinic. One hundred fifty-nine clinically diagnosed CP patients and 83 PDAC patients were enrolled in this study from Ogaki Municipal Hospital, Japan Community Health Care Organization Osaka Hospital, and Osaka University Hospital. The diagnosis of CP was made according to the guidelines of the Japan Pancreas Society [19]. Sera from these subjects were collected and kept frozen at −80 °C until use.

Laboratory measurements

Serum biochemical variables [aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), albumin (Alb), total bilirubin (T-Bil), creatinine (Cr), total cholesterol (T-Chol), triglyceride (TG), C-reactive protein (CRP), amylase (AMY)] were measured with a conventional automated analyzer. Serum levels of carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) were determined using a chemiluminescent enzyme immunoassay.

Lectin-antibody ELISA for Fuc-Hpt

Lectin-antibody ELISA for Fuc-Hpt (AAL-Hpt, PhoSL-Hpt) was performed as described previously [22]. Briefly, the bottom of a 96-well ELISA plate was coated with the Fab fragment of anti-human Hpt IgG (Dako, Carpinteria, CA), because IgG has fucosylated oligosaccharides in its Fc portion. Coated plates were blocked with phosphate-buffered saline (PBS) containing 3% bovine serum albumin for 1 h, followed by washing with PBS containing 0.1% Tween 20 (PBS-T). A 2-μl aliquot of serum was placed into each well and incubated for 1 h at room temperature. The plate was washed three times with PBS-T, using Immuno Wash (Bio-RAD Model 1517, Bio-RAD, Tokyo, Japan). To detect Fuc-Hpt, biotinylated AAL or PhoSL diluted 1:1000 was placed into each well, followed by incubation at room temperature for 1 h. After washing the plates three times with PBS-T, peroxidase-conjugated avidin was added to each well, followed by incubation at room temperature for 1 h. After washing four times with PBS-T, tetramethylbenzidine was added to each well, followed by a 15-min incubation for development. To stop the development, 1 N sulfuric acid was added to each well. A standard curve for Fuc-Hpt was obtained as previously described [20] using conditioned medium from the pancreatic cancer cell line PK8 that was transfected with an expression vector for Hpt (Takara Bio Inc., Shiga, Japan). We previously investigated the efficacy and reproducibility of our developed ELISA kits [11,12,21].

Statistical analysis

Statistical analysis was conducted using JMP Pro 11.0 software (SAS Institute Inc., Cary, NC). Variables were expressed as the mean ± standard deviation (SD). Statistical analysis included descriptive statistics, analysis of variance, the Wilcoxon and Kruskal–Wallis tests, and Spearman R correlations. The diagnostic performances of the scoring systems were assessed by analyzing receiver operating characteristic (ROC) curves. The probabilities of true positive (sensitivity) and true negative (specificity) assessments were determined for selected cut-off values, and the area under the ROC curve (AUROC) was calculated for each index. The Youden index was used to identify the optimal cut-off points. Differences were considered statistically significant at $P < 0.05$.

Results

Serum biochemical variables and Fuc-Hpt levels in study subjects

The descriptive characteristics of the study subjects are shown in Table 1. The average age, serum AST, ALT, GGT, ALP, TG, CRP, CA19-9, and CEA were significantly lower in HV subjects than in CP and PDAC patients. Serum AMY levels were significantly higher in CP patients than in HV and PDAC participants. Serum AAL-Hpt levels