Original Article

Endosonographic features in patients with non-alcoholic early chronic pancreatitis improved with treatment at one year follow up

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Since the prevention of early chronic pancreatitis (ECP) into chronic pancreatitis might be critical for the reduction of pancreatic cancer, we tried to clarify the pathophysiology of ECP patients, focusing on ECP patients without alcoholic chronic pancreatitis. 27 ECP patients without alcoholic chronic pancreatitis and 33 patients with functional dyspepsia with pancreatic enzyme abnormalities (FD-P) were enrolled in this study. Diagnosis of ECP was made when imaging findings showed the presence of more than 2 out of 7 endoscopic ultrasound features. Duodenal degranulated eosinophils and glucagon-like peptide 1 producing cells were estimated by immunostaining. There were no significant differences in characteristics and psychogenic factors between ECP and FD-P patients. Interestingly, endoscopic ultrasound score in ECP patients significantly improved, albeit clinical symptoms in ECP patients showed no improvement at one year follow up. The extent of migration of duodenal degranulated eosinophils in FD-P patients was significantly higher compared to that in ECP patients. The levels of elastase-1 and trypsin in ECP patients with improved endoscopic ultrasound features were significantly reduced by the treatment. Further studies will be needed to clarify whether clinical symptoms and endoscopic ultrasound features in ECP patients without alcoholic chronic pancreatitis were improved in longer follow up study.

Key Words: **early chronic pancreatitis, functional dyspepsia, pancreatic enzyme abnormalities, endosonography, degranulated eosinophil**

Functional gastrointestinal disorder (FGID) has been shown to overlap with each other[1] and the numbers of patients with FGID have increased. There are some reports in which chronic pancreatitis is associated with functional dyspepsia (FD), and we have already reported that there were early chronic pancreatitis (ECP) patients among patients with refractory epigastric pain.[2] ECP is considered to be early stage of chronic pancreatitis and exhibits symptoms similar to FD in Japan.[2,3] Since epigastric pain in ECP patients was improved by the treatment for chronic pancreatitis and aggravated by therapeutics targeting of FD, a precise diagnosis of ECP is critical to control clinical symptoms in ECP patients and to prevent this disease from advancing into chronic pancreatitis.[4] There were no available data about the differences between ECP and functional dyspepsia with pancreatic enzyme abnormalities (FD-P) patients. The prevention of ECP into chronic pancreatitis might be critical for the reduction of pancreatic cancer.[5] We have previously reported that epigastric pain in ECP patients can be improved with a combination therapy consisting of camostat mesilate, pancrelipase and rabeprazole, in contrast, the treatment of FD with a combination therapy of acotiamide and proton pump inhibitors aggravated epigastric pain in ECP patients.[6]

Masamune *et al.*[7] have reported that alcohol-related ECP patients have tendency to promote to the aggravation of endoscopic ultrasound (EUS) scores. However, there were not precise data about ECP patients without alcoholic chronic pancreatitis. In addition, there were no available data to differentiate ECP patients without alcoholic chronic pancreatitis from patients with FD-P. Therefore, in this study, we tried to clarify differences in the pathophysiology including duodenal responses such as degranulated eosinophils and glucagon-like peptide 1 (GLP-1) productive cells associated with gastric emptying[8,9] between ECP patients, especially ECP patients without alcoholic chronic pancreatitis and FD-P patients. In addition, we aimed to evaluate the efficacy of treatment of ECP patients at one year follow up in view of clinical symptoms, pancreatic enzymes and EUS features.

Materials and Methods

Patients. From April 2015 to April 2018, all patients who presented with refractory epigastric pain of unknown etiology at Nippon Medical School Hospital and Nippon Medical School Musashi Kosugi Hospital were evaluated for enrollment in this study. Among them, there were 80 patients with at least one abnormal value in pancreatic enzymes, and 60 patients of them underwent EUS. Finally, 27 patients meeting the diagnostic criteria of ECP and 33 patients with FD-P were enrolled as described in Fig. 1. ECP was classified as alcoholic chronic pancreatitis when daily alcohol consumption was >60 g for females and >80 g for males per day during a period of at least 2 years. Five different pancreatic enzymes in the sera of patients were measured, including amylase, lipase, elastase-1, trypsin, and phospholipase A2 (PLA2). FD patients with more than one pancreatic enzyme abnormality were classified as FD-P (Fig. 1). Abnormal values for pancreatic enzymes were defined as follows: amylase >129 U/L, lipase >58 U/L, elastase-1 >301 ng/dl, trypsin >551 ng/ml, and PLA2 >401 ng/dl. Exclusion criteria included severe systemic illness and history of malignant diseases. We evaluated pancreatic enzymes at one year posttreatment and compared their levels between treatment interventions. Written informed consent was obtained from subjects prior to undergoing

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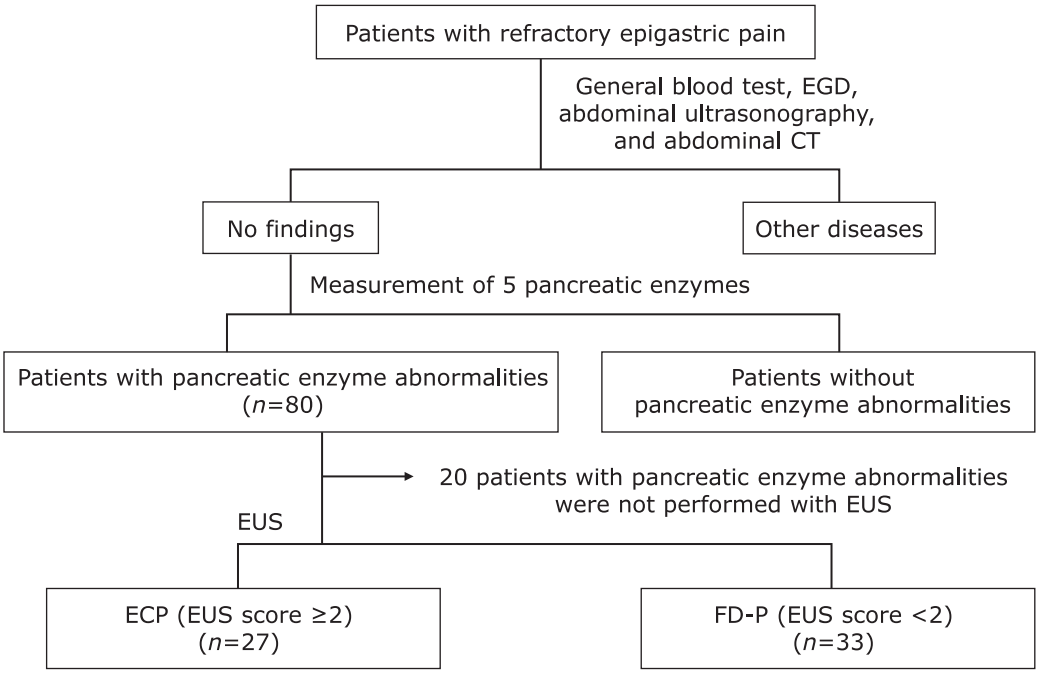

Fig. 1. Study protocol.

EUS. The study protocol was approved by the Ethics Review Committee of Nippon Medical School Hospital.

Evaluation of clinical symptoms. Clinical symptoms of enrolled patients were evaluated according to the Rome III criteria.[10] Clinical symptoms must have been at least one of the following: early satiation, bothersome postprandial fullness, epigastric pain or epigastric burning. FD symptoms were evaluated as follows: 0, none; 1, very mild; 2, mild; 3, moderate; 4, severe; 5, very severe. Clinical symptoms were evaluated with the Gastrointestinal Symptom Rating Scale (GSRS).[11] We used the mean score of the GSRS and the 15 gastrointestinal symptoms of the GSRS for the evaluation of dyspeptic symptoms.

Self-rating questionnaire for depression (SRQ-D). Status of depression was evaluated using self-rating questionnaire for depression. The SRQ-D comprises 18 items, which are rated on a 4-point scale (0, no; 1, sometimes; 2, frequently; 3, always). In this study, we determined patients having SRQ-D scores of 16 points or more as having symptoms of depression.[12]

Health-related quality of life (HRQOL). The Social Functioning-8 (SF-8) test was used to measure health-related quality of life according to the Manual of the SF-8 Japanese Version.[13]

Pittsburgh Sleep Quality Index (PSQI). Sleep quality and sleep duration were evaluated by a Japanese version of the Pittsburgh Sleep Quality Index (PSQI) questionnaire. Higher scores indicate poorer sleep.[14,15] A cut-off score >5.5 has a sensitivity of 80.0–85.7% for various patient groups and a specificity of 86.6% for control subjects in the Japanese version of the PSQI.[14,15]

Measurement of gastric emptying. The half gastric emptying time ($T_{1/2}$; min) and the lag phase (T_{max}; min) were measured according to Hellmig *et al.*[16] T_{max} value greater than 60 min, representing the mean T_{max} in healthy volunteers plus SD, was defined to represent relative disturbances in gastric emptying according to the diagnostic criteria of the Japan Society of Smooth Muscle Research and our own study.[17,18]

Scoring of EUS. An Olympus EUS-UCT 260 convex scanning endosonography (Olympus America, Melville, NY) at 7.5 MHz was used to perform EUS under conscious sedation. In this study, diagnostic features of EUS conformed to the diagnostic criteria according to Evidence-based Clinical Practice Guidelines for Chronic Pancreatitis 2015 in Japan. Diagnosis of ECP can be made by imaging findings as more than two among the seven features including any of (1)–(4) [(1) lobularity with honeycombing, (2) lobularity without honeycombing, (3) hyperechoic foci without shadowing, (4) stranding, (5) cysts, (6) dilated side branches, and (7) hyperechoic MPD margin] and clinical findings as two or more than items among repeated attacks of upper abdominal pain, abnormalities in blood or urine pancreatic enzymes, exocrine pancreatic dysfunction and persistent drinking history (80 g/day).[19] The total number of these seven items was used as an index of the EUS score. EUS features were evaluated and confirmed by three expert endoscopists including at least a pancreatic specialist (Japan Pancreas Society) on conducting EUS survey in a blind manner. When opinions differed among expert endoscopists, a final judgement was arrived at by consensus following a discussion of each individual case.

Duodenal inflammatory cells and GLP-1-positive cells infiltration. Histological duodenitis was assessed according to criteria of previous studies[20] and modified criteria of a previous report.[21] Specimens were evaluated by two experienced pathologists in a blinded manner. GLP-1 positive cells or degranulated eosinophils were determined by rabbit anti-GLP-1 antibody (Abcam, Cambridge, UK; 1:2,000) and mouse anti-PRG2 antibody (Gene Tex, California, CA; 1:50), respectively.

Treatment. The treatment regimen for patients consisted of at least 12 weeks of a triple therapy comprising a proton pump inhibitor (PPI), camostat mesilate (300 mg/day), and pancrelipase (1,200 mg/day). Study participants were instructed to refrain from consuming fatty meals.

Data Analysis. We determined the area under the curve at 5 min (AUC_5) and at 15 min (AUC_{15}) values as markers of the early phase of gastric emptying based on previous studies.[18,22]

Statistical analysis. The χ^2 test and Fisher's exact test were used to compare categorical variables, and the Mann-Whitney *U* test was used to compare continuous variables for univariate analysis between two groups. Comparisons of continuous variables between before and after treatment were performed using the

Table 1. Characteristics of ECP and FD-P patients

	ECP (n = 27)	FD-P (n = 33)	p value
Age (year old)	57.07 ± 14.45	60.24 ± 15.07	0.41
Sex (M:F)	10:17	6:27	0.18
Alcohol consumption (g/day)	5.65 ± 8.40	14.04 ± 48.01	0.45
>80 g/day for male (n)	0	0	N/A
>60 g/day for female (n)	0	0	N/A
Smoking (Brinkman index)	179.76 ± 231.48	142.2 ± 354.2	0.11
GSRS	2.48 ± 1.23	2.58 ± 0.76	0.24
Epigastric pain	3.00 ± 1.37	2.88 ± 1.27	0.74
Epigastric burning	2.00 ± 1.15	2.44 ± 1.04	0.13
Early satiety	2.95 ± 1.22	2.92 ± 1.32	0.92
Postpradial fulness	3.79 ± 0.98	3.80 ± 0.87	0.83

Smoking was evaluated using Brinkman index. ECP, early chronic pancreatitis; FD-P, functional dyspepsia with pancreatic enzyme abnormalities; GSRS, Gastrointestinal Symptom Rating Scale; N/A, not available.

Table 2. Comparison of global PSQI, SF-8 and SRQ-D scores between ECP and FD-P patients

Factors	ECP (n = 27)	FD-P (n = 33)	p value
Global-PSQI	3.79 ± 1.62	5.24 ± 2.86	0.08
PCS	45.11 ± 7.11	43.77 ± 7.92	0.36
MCS	44.59 ± 7.77	43.05 ± 7.95	0.42
SRQ-D	10.79 ± 3.74	12.28 ± 5.64	0.37

ECP, early chronic pancreatitis; FD-P, functional dyspepsia with pancreatic enzyme abnormalities; PSQI, Pittsburgh Sleep Quality Index; PCS, physical component summary; MCS, mental component summary; SRQ-D, Self-Rating Questionnaire for Depression.

Table 3. Comparison of gastric motility between ECP and FD-P patients

	ECP (n = 27)	FD-P (n = 33)	p value
T_{max}	64.23 ± 31.08	60.29 ± 22.52	0.248
$T_{1/2}$	109.21 ± 87.25	95.49 ± 49.15	0.239
AUC_5	20.81 ± 7.95	22.47 ± 5.90	0.492
AUC_{15}	49.8 ± 16.37	51.65 ± 9.98	0.478

ECP, early chronic pancreatitis; FD-P, functional dyspepsia with pancreatic enzyme abnormalities; T_{max}, the lag phase as the point of maximum gastric emptying; $T_{1/2}$, the half gastric emptying; AUC, area under the curve.

Wilcoxon signed-rank test. Statistical analysis was performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R ver. 2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, EZR is a modified version of R commander (ver. 1.6-3) designed to add statistical functions frequently used in biostatistics. Differences between variables with $p<0.05$ were considered to be statistically significant.

Results

Characteristics of ECP and FD-P patients. We evaluated clinical characteristics of ECP and FD-P patients (Table 1). Age, sex and GSRS score did not differ statistically between ECP (n = 27) and FD-P patients (n = 33) (Table 1). Differences in FD symptoms, including epigastric pain, epigastric burning, early satiety and postprandial fullness did not vary significantly between ECP and FD-P patients (Table 1).

Comparison of global PSQI, SF-8 and SRQ-D scores between ECP and FD-P patients. To compare psychogenic factors such as depression between ECP and FD-P patients, we evaluated SRQ-D scores for the two groups. There were no statistically significant differences in SRQ-D scores between the two groups (Table 2). There was also no statistically significant difference in global PSQI scores between two groups (Table 2). In addition, to compare quality of life (QOL) between the two groups, we estimated PCS and MCS between two groups. There were also no statistically significant differences in MCS and PCS between the two groups (Table 2).

Comparison of gastric motility between ECP and FD-P patients. There were not any significant differences in T_{max} and $T_{1/2}$ values between ECP and FD-P patients (Table 3). In addition, AUC_5 and AUC_{15} values representing early gastric emptying in ECP patients did not differ significantly from those in FD-P patients (Table 3).

Comparison of degranulated eosinophils and GLP-1 producing cells in duodenal mucosa in ECP patients with those in FD-P patients. Since studies have shown the migration of eosinophils into the duodenal mucosa of FD patients, we examined whether the degree of infiltrations of degranulated eosinophils into the duodenum differed between FD-P and ECP patients. Since GLP-1 secretion is associated with gut motility, we compared the numbers of duodenal GLP-1 producing cells between the two groups. There were significant (p = 0.0017 and $p<0.01$, respectively) differences in the migrations of duodenal GLP-1 producing cells and degranulated eosinophils between these distinct groups (Fig. 2A and C & Fig. 2B and C).

Since studies have shown that GLP-1 modified gastric motility, we investigated the correlation between the number of duodenal GLP-1 positive cells and gastric emptying parameters such as T_{max}, AUC_5 and AUC_{15} values in ECP patients and FD-P patients. The number of GLP-1 positive cells were not significantly (p = 0.861, R = –0.043; p = 0.414, R = 0.199; p = 0.436, R = 0.19, respectively) associated with T_{max}, AUC_5 or AUC_{15} values in ECP patients. In addition, the number of GLP-1 positive cells showed no statistically significantly correlation with T_{max}, AUC_5 or AUC_{15} values in FD-P patients (p = 0.323, R = –0.298; p = 0.962, R = –0.0148; p = 0.96, R = 0.0154, respectively).

EUS scores in ECP and FD-P patients at pretreatment vs posttreatment. EUS scores in ECP patients (2.43 ± 0.60) were significantly ($p<0.01$) higher compared to those (0.33 ± 0.48) in FD-P patients (Fig. 3A, B and D). To clarify whether a 12-week treatment regimen improved EUS features in ECP patients and FD-P patients at one year follow up, we compared the scoring of EUS features between the two groups. EUS scores in ECP patients (2.43 ± 0.60) significantly (p = 0.0229) improved after

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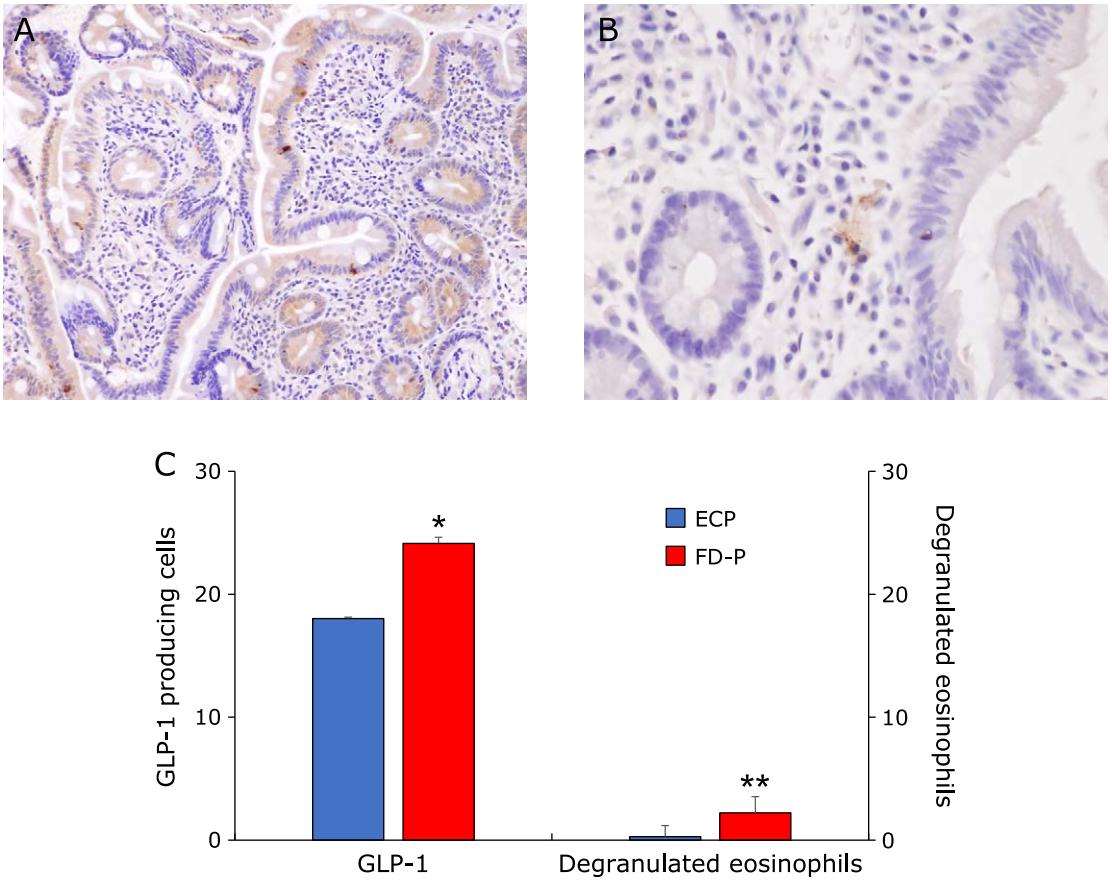

Fig. 2. Comparison of duodenal degranulated eosinophils and GLP-1 producing cells in ECP and FD-P patients. (A) Immunostaining of GLP-1 producing cells in the duodenal mucosa of ECP patients (magnification ×200). (B) Immunostaining of degranulated eosinophils in the duodenal mucosa of ECP patients (magnification ×400). (C) Migration of degranulated eosinophils in the duodenum of patients with FD-P was significantly higher ($p = 0.0087$) compared to that in ECP patients. Infiltrations of GLP-1 producing cells in the duodenum of patients with FD-P was significantly higher ($p = 0.0017$) compared to ECP patients. *$p = 0.0017$ and **$p = 0.0087$ vs ECP.

the treatment (1.86 ± 0.96) (Fig. 3B and D). There were 10 subjects (47.6%) who had improved total EUS scores at one year follow up, 8 (38.1%) with no change in scores, and 3 (14.3%) with worse score. Lobularity with honeycombing and hyperechoic MPD margin in ECP patients improved significantly ($p = 0.048$ and $p = 0.03$, respectively) in ECP patients who also showed improved EUS scores compared to ECP patients with no improvement in EUS score (Table 4). Although three EUS features (Lobularity with honeycombing, dilated side branches, and hyperechoic MPD margin) fully recovered in all ECP patients with improved EUS scores, improvement in two other EUS features (hyperechoic foci without shadowing and stranding) was seen in less than half of ECP patients (Table 4). Alcohol consumption in ECP patients with aggravated EUS scores (13.2 ± 12.8 g/day) was significantly higher than other two groups (2.1 ± 3.4 g/day or 4.1 ± 3.4 g/day) ($p = 0.046$) (Table 4).

Comparison of clinical symptoms in ECP patients after the treatment. GSRS score in ECP patients at one year follow up showed no statistically significantly improvement compared to pretreatment scores (Fig. 3D). There were not significant differences in GSRS score between ECP and FD-P patients (Fig. 3D). GSRS in ECP patients did not differ significantly ($p = 0.941$) between pretreatment and posttreatment (Fig. 4). There was no significant ($p = 0.582$) difference in GSRS of ECP patients showing no improvement in EUS score (no change or aggravated group) at pretreatment (2.81 ± 1.69) vs posttreatment (2.46 ± 0.85).

Pancreatic enzymes in ECP patients with and without improvement in EUS features at one year follow up. Lipase and PLA2 levels in ECP patients were significantly ($p = 0.040$ in both) higher compared to those in FD-P patients (Fig. 5). There were no statistically significantly differences in amylase, elastase-1 and trypsin levels between ECP and FD-P patients (Fig. 5). Then, the levels of elastase-1 and trypsin in ECP patients with improved EUS score at one year follow up were significantly ($p = 0.01$ and $p<0.01$, respectively) lower after treatment (Fig. 6A). In contrast, treatment failed to ameliorate pancreatic enzymes in ECP patients with no improvement in EUS score at one year follow up (Fig. 6B).

Discussion

The major findings of this study are: 1) EUS score in ECP patients was significantly improved after the treatment in one year follow up, albeit GSRS in ECP patients was not ameliorated, 2) migration of duodenal degranulated eosinophils in FD-P patients was significantly higher compared to ECP patients, 3) lipase and PLA2 levels in ECP patients were significantly higher than in FD-P patients, and 4) treatment improved elastase-1 and trypsin serum levels in patients with improved EUS score.

Since chronic pancreatitis has a high mortality rate due to a variety of factors, it is critical to identify chronic pancreatitis at its early stage.[23,24] Some studies suggest that dyspeptic symptoms and symptoms consisting with irritable bowel syndrome may be

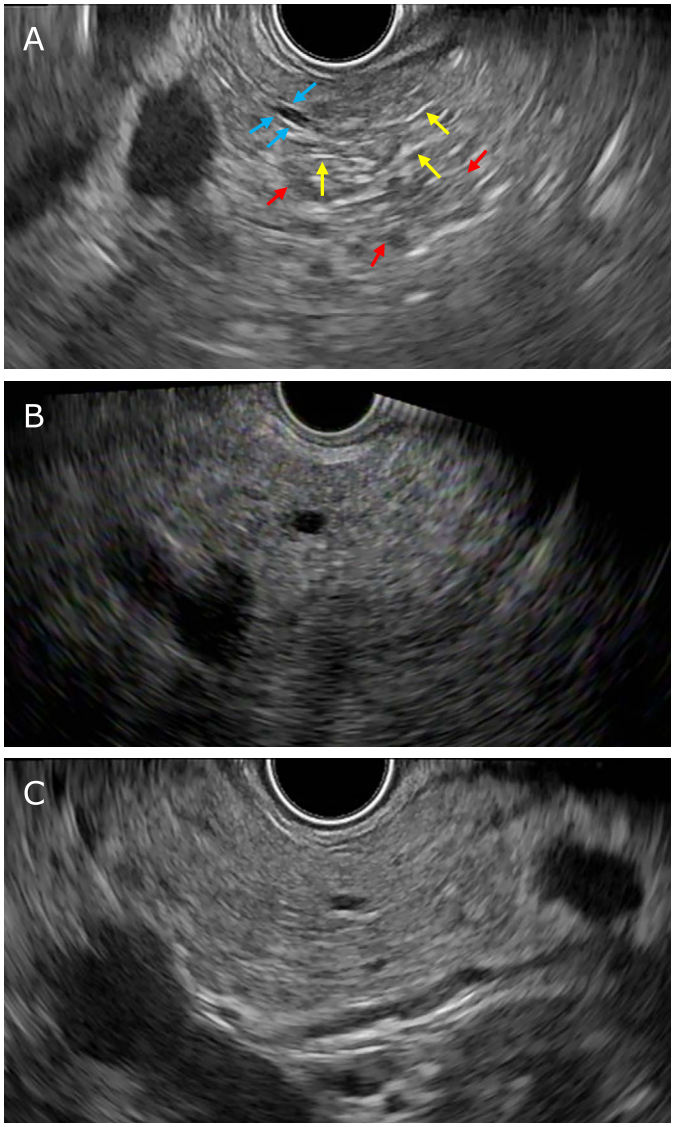

Fig. 3. EUS scores in ECP and FD-P patients between pretreatment and posttreatment. (A) Representative EUS features of ECP patients at pretreatment. There were three EUS features (lobularity without honeycombing, hyperechoic foci without shadowing and hyperechoic MPD margin). Red arrows show lobularity without honeycombing. Yellow arrows show hyperechoic foci without shadowing. Blue arrows show hyperechoic MPD margin. (B) Representative EUS features of ECP patients at posttreatment. EUS features identified at pretreatment disappeared. Fig. 3 (A) and (B) are images from the same patients. (C) Representative EUS features of FD-P patients. There was none or one of abnormal findings of EUS features in FD-P patients. (D) EUS score of ECP patients at posttreatment significantly ($p = 0.0229$) improved compared to that in ECP patients in pretreatment. GSRS score in ECP patients at one year follow up study did not significantly improve compared to pretreatment. *$p<0.01$ vs FD-P, **$p = 0.0229$ vs pretreatment ECP.

the first and single clinical manifestation of chronic pancreatitis.[25,26] Although it is very important to differentiate chronic pancreatitis from FD-P, our study shows no significant difference in clinical characteristics, psychogenic factors and FD symptoms between these two cohorts of patients. Therefore, we tried to determine whether there were any differences between ECP and FD-P patients at one year follow up. In this study, clinical symptoms were refractory to treatment of chronic pancreatitis in both of ECP patients and FD-P patients as shown in Fig. 3. Masamune *et al.*[7] have reported that most of ECP patients were not advanced in two years follow up. Considering that others have also found that in the majority of patients with ECP did not fully develop into CP at two year follow up,[7] one year follow up might not be sufficient time to differentiate ECP from FD-P patients. In addition, considering that two FD-P patients advanced into ECP patients in this study, certain FD-P patients (2/33) may be early stage of ECP patients.

Since it is generally accepted that the mechanism of pancreatitis is premature activation of digestive enzymes within the acinar cell leading to autodigestion,[27] we measured five different pancreatic enzymes in this study to determine whether elastase-1 and PLA2 levels in ECP patients may predict the improvement of EUS features in response to treatment. Then, the measurement of elastase-1 and PLA2 levels in ECP patients may predict the improvement for EUS features as the responsiveness to the treatment. Feinle-Bisset *et al.*[28] have shown that the manner in which fat is absorbed depends on the acylchain length, which may explain in part the fact that fat droplet size also affects gut function and clinical symptoms. There is evidence that fat emulsion droplet size is also a key physicochemical variable affecting gastrointestinal function and satiety.[29–31] The droplet size effect can be explained by the fact that, for a given mass of fat, a smaller droplet size offers a larger lipid surface area.[32] The increased surface area will, in turn, allow a higher number of lipase molecules to bind at the oil-water interface because excess lipase exert on them.[33,34] This excessive secretion of lipase may lead to chronic pancreatitis. Therefore, a reduction in lipase levels may reflect a suppression of excessive secretion via the amelioration of pancreatitis or droplet size. In addition, Sitaraman *et al.*[35] have reported that elevations of serum amylase or lipase were associated with chronic pancreatitis, biliary stones, and biliary sludge. Therefore, the etiology of ECP patients without alcoholic chronic pancreatitis may be associated with fat droplet size in the gut lumen, biliary stones, biliary sludge and transition from acute pancreatitis to ECP.[35–37] Moreover, the elevation of pancreatic enzymes in early chronic pancreatitis are believed to be due to acinar cell damage, however, these values can fluctuate and thus normal levels of amylase or lipase do not necessarily exclude chronic pancreatitis.[38] Further studies will be needed to clarify whether elastase-1 and trypsin levels are indeed useful markers for improvement in ECP patients as described in Fig. 6.

In our data, EUS score in ECP patients significantly improved compared to the pretreatment. Considering that Masamune *et al.*[7] have also reported that aggravated ECP patients were all ECP patients taking more than 80 g/day alcohol consumption, ECP patients without alcoholic chronic pancreatitis may well respond to treatment. To focus on which EUS features in ECP patients without alcoholic chronic pancreatitis were ameliorated by the treatments, we compared seven EUS features between pretreatment and posttreatment. In our data, hyperechoic MPD margin, lobularity and dilated side branches vanished with the treatments. Huang *et al.*[39] have also reported that hyperechoic MPD margin represents the state of long-standing luminal increased pressure. In addition, dilated side branches occur, partially forming focal tiny cysts. Masamune *et al.*[7] have also reported that 51% of idiopathic ECP patients were downgraded at two-year follow up. Considering previous study and our data, it might be possible to prevent the advance of ECP into chronic pancreatitis, if possible, before fibrosis or stranding begins to develop in pancreatic tissue. In contrast, Yamabe *et al.*[37] and Rajan *et al.*[40] have reported that changes in EUS features can be nonspecific and seen in healthy patients. Ikeda *et al.*[41] and Petrone *et al.*[42] have demonstrated that advanced age was also significantly associated with an increased risk of MPD dilatation.

Duodenal inflammation has been linked to FD.[43–45] Eosinophilic infiltration of duodenal mucosa has been found to increase significantly in patients with FD compared to controls.[46–48] In another study, Vanheel *et al.*[43] discovered that not only eosino-

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Table 4. Comparison of EUS scores in ECP and FD-P patients between pretreatment and posttreatment

	ECP with improved EUS score	ECP with unimproved EUS score	*p* value
Age	58.4 ± 15.9	61.9 ± 12.9	0.649
Sex (M:F)	5:05	5:06	1
Alcohol consumption (g/day)	2.1 ± 3.4	7.1 ± 8.6	0.179
		4.1 ± 3.4 (no change) / 13.2 ± 12.8† (aggravated)	
GSRS	2.22 ± 0.73	2.81 ± 1.69	0.796
EUS score			
Lobularity with honeycombing	5/5 (100%)	0/2 (0%)	0.048
Lobularity without honeycombing	N/A	0/1 (0%)	N/A
Hyperechoic foci without shadowing	1/4 (25.0%)	0/4 (0%)	1
Stranding	3/9 (33.3%)	1/9 (11.1%)	0.576
Cysts	N/A	N/A	N/A
Dilated side brenches	2/2 (100%)	2/2 (100%)	1
Hyperechoic MPD margin	6/6 (100%)	1/4 (25%)	0.033

†$p = 0.046$ vs ECP patients with improved EUS score or ECP patients without change of EUS score. ECP, early chronic pancreatitis; FD-P, functional dyspepsia with pancreatic enzyme abnormalities; EUS, endosonography; GSRS, Gastrointestinal Symptom Rating Scale; MPD, middle pancreatic duct; N/A, not available.

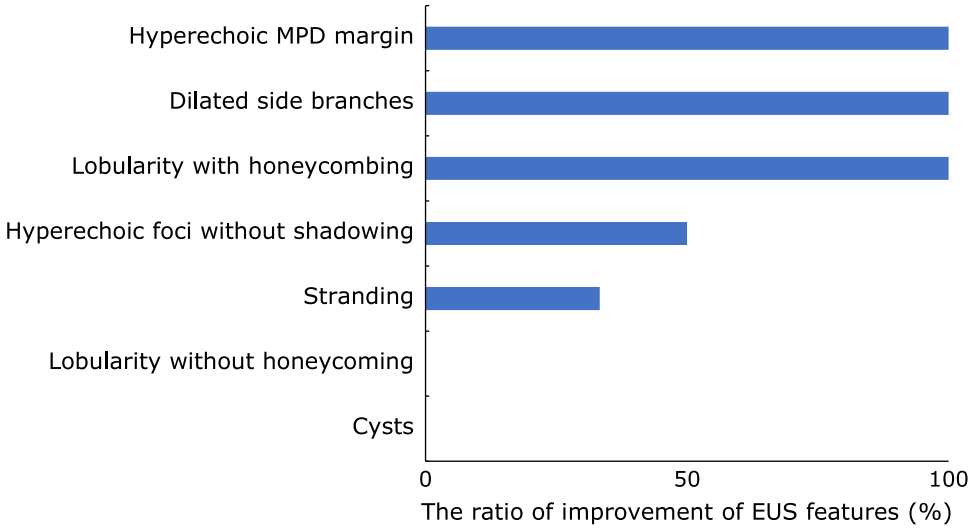

Fig. 4. Ratio of the improvement of EUS features in ameliorated ECP patients. All three EUS features (lobularity with honeycombing, dilated side branches, and hyperechoic MPD margin) fully recovered in all ameliorated ECP patients.

phils, but also mast cells, were found to be increased in the duodenum of patients with FD. Interestingly, infiltration of eosinophils has only been associated with eosinophil degranulation in the GI tract, and eosinophil granules, such as major basic protein (MBP), an eosinophil cationic protein are cytotoxic to a variety of tissues, including the intestinal epithelium.[49] Our study found significant migration of duodenal degranulated eosinophils in the tissue of FD-P patients compared to ECP patients. Further studies will be needed to clarify how degranulated eosinophils migrations affect the differences in the pathophysiology between ECP and FD-P patients. In addition, considering the disturbance of gastric emptying in the two groups and the association between GLP-1 production and the inhibition of gastric emptying,[50,51] we compared infiltrations of GLP-1 producing cells in the duodenum between FD patients and ECP patients. In this study, the numbers of GLP-1 producing cells in the duodenum of ECP patients were significantly lower compared to those in FD-P patients, corresponding to a previous report.[38] We could not clarify why infiltration of degranulated eosinophils associated with an increase in GLP-1 producing cells in the duodenum. Bang-Berthelsen *et al.*[52] have reported that GLP-1 induced IL-33 production, and Angulo *et al.*[53] have also reported that IL-33 induced eosinophil activation. In addition, Johnston *et al.*[54] have also shown that IL-33 in local tissues contributes to degranulated eosinophil infiltration. Further studies will be needed to clarify whether degranulated eosinophil infiltration into the duodenum induces an increase in duodenal GLP-1 producing cells through the upregulation of IL-33 production.

Taken together, no significant differences in clinical characteristics were found between ECP and FD-P patients. Accumulations of duodenal degranulated eosinophils might contribute to differences in the pathophysiology between ECP and FD-P patients. However, EUS scoring in ECP patients without alcoholic chronic pancreatitis significantly improved compared to pretreatment. Further studies are essential to clarify the role of duodenal degranulated eosinophils in the pathophysiology of ECP and FD-P patients and the correlation between abnormalities in pancreatic enzymes and response to treatment as evaluated by EUS scoring.

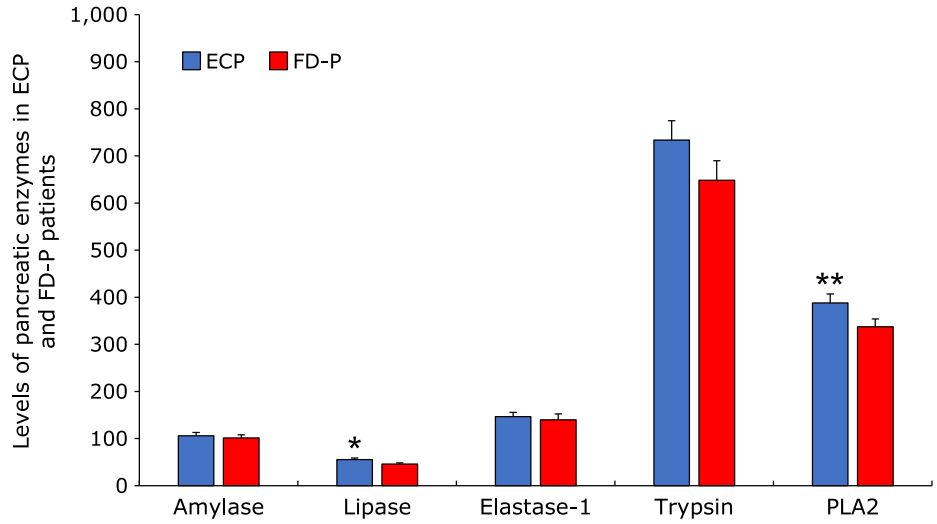

Fig. 5. Levels of pancreatic enzymes in ECP and FD-P patients. Lipase (U/L) and PLA2 (ng/dl) levels in ECP patients were significantly ($p = 0.040$ in both) higher compared to FD-P patients. There were no significant differences in amylase (U/L), elastase-1 (ng/dl) and trypsin (ng/ml) levels between ECP and FD-P patients. *$p = 0.0395$ and **$p = 0.0389$ vs FD-P.

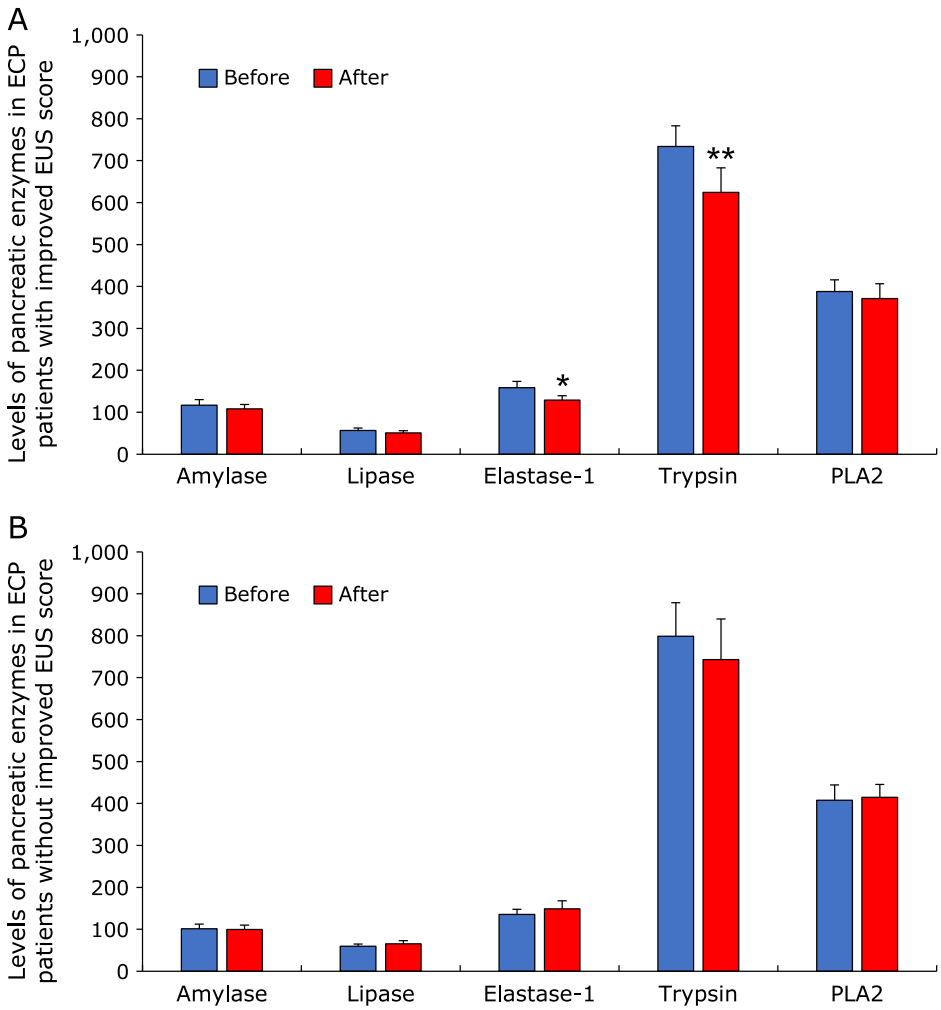

Fig. 6. Pancreatic enzymes in ECP with and without improved EUS features at one year follow up. (A) The levels of elastase-1 and trypsin in ECP patients with improved EUS score at one year follow up were significantly ($p = 0.01$ and $p<0.01$, respectively) reduced with treatment. *$p = 0.0143$ vs the level of elastase-1 in ECP patients after treatment, **$p = 0.0389$ vs the level of trypsin in ECP patients after treatment. (B) In contrast, no pancreatic enzymes were improved by the treatment in ECP patients whose EUS score had not improved at one year follow up.

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Author Contributions

KH: data collection, writing the manuscript; HY, MM, KK, SA, GI, HN, YK, NU, KKaneko, RO, and KI: data collection; KG: editing, revising manuscript, SF: data collection, direction of experiment, writing the manuscript.

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Conflicts of Interest

No potential conflicts of interest were disclosed.

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