



CLINICAL RESEARCH

Fractalkine and TGF- β 1 levels reflect the severity of chronic pancreatitis in humans

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Abstract

AIM: To clarify whether serum chemokine and cytokine levels can become useful biological and functional markers to assess the severity of chronic pancreatitis (CP). This study aimed at clarifying whether serum chemokine and cytokine levels can become useful biological and functional markers to assess the severity of CP.

METHODS: Serum monocyte chemoattractant protein-1 (MCP-1), transforming growth factor beta-1 (TGF- β 1), and soluble type fractalkine (s-fractalkine) concentrations were examined in patients with CP ($n = 109$) and healthy controls ($n = 116$). Severity of disease was classified in patients with CP by a staging system. Relationships between stage-specific various clinical factors and serum MCP-1, TGF- β 1, and s-fractalkine levels were investigated. Furthermore, 57 patients with non-alcoholic CP were similarly evaluated in order to exclude influence of alcohol intake.

RESULTS: Patients with CP showed significant higher levels of serum TGF- β 1 and s-fractalkine, but not MCP-1, compared to the controls. Serum TGF- β 1 in the severe stage and s-fractalkine in the mild and the

severe stage of CP significantly increased compared to those of controls. However, it was observed that both TGF- β 1 and s-fractalkine levels were affected by alcohol intake. In patients with non-alcoholic CP, serum TGF- β 1 showed significant increase in the moderate stage of CP, and serum s-fractalkine revealed significant increase in the early stage of CP. **CONCLUSION:** It is suggested that the measurement of serum F-fractalkine is useful to diagnose early-stage CP. Moreover, the combined determination of both, s-fractalkine and TGF- β 1, in human sera may be helpful in evaluating the severity status of CP.

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Key words: Chronic pancreatitis; Transforming growth factor beta-1; Soluble fractalkine; Monocyte chemoattractant protein-1

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INTRODUCTION

Chronic pancreatitis (CP) is a chronic clinical disorder characterized by irreversible damage to the pancreas, the development of histologic evidence of inflammation and fibrosis, and eventually the destruction and permanent loss of exocrine and endocrine tissue^[1-4]. Furthermore, patients with long-standing CP are also at a markedly increased risk of developing pancreatic cancer^[3]. Unfortunately, simple, indirect measurements of decreased pancreatic function do not show abnormality until CP is considerably advanced. Imaging or function tests may not reveal early CP, and the results of these tests do not necessarily correlate with each other^[5-9]. The quest continues for useful biological and functional markers of early-stage CP. Recently, chemokines and

cytokines have been recognized to be important factors in the progression of CP^[10,11]. A preliminary study by us in a small number of CP patients showed the possibility that measurement of serum soluble type fractalkine (s-fractalkine) may be useful for diagnosing early-stage of CP^[7]. Thus, in the current study, using a large number of CP patients classified by severity with a staging system, we investigated whether chemokine and cytokine levels can become useful biological and functional markers of early-stage CP, focusing particularly on monocyte chemoattractant protein-1 (MCP-1), transforming growth factor beta-1 (TGF- β 1), and s-fractalkine, which are supposed to be involved in chronic inflammation.

MATERIALS AND METHODS

Patients

One hundred nine patients with CP (63 males and 46 females; age range, 25–81 years; mean, 56.8 years) who fulfilled clinical diagnostic criteria for CP by the Japan Pancreas Society^[12] and 116 healthy controls (69 males and 47 females; age range, 26–93 years; mean, 56.5 years) were selected for this study. Patients and healthy controls with recent inflammatory diseases, e.g. infectious diseases, chronic hepatitis, or substantial alcohol consumption were excluded. The study protocol was approved by the ethics committee at Kyushu University.

CP patients were classified according to a staging system reported previously (Table 1)^[12–14]. The staging system comprises 6 parameters: exocrine pancreatic function (scored 0–3), pancreatic imaging tests by endoscopic retrograde cholangiopancreatography (ERCP; scored 0–4), glucose metabolism (scored 0–4), pain (scored 0–4), alcohol intake (scored 0–2), and complications associated with CP (scored 0–2). The CP is then subclassified, according to a total score from these 6 grading factors, into mild (total score, 0–3), moderate (total score, 4–7), and severe (total score, \geq 8). The number of patients with mild, moderate, or severe disease was 36 (33.0%), 49 (45.0%), and 24 (22.0%), respectively (Table 2). We analyzed whether each grading factors and severity of CP are related with serum MCP-1, TGF- β 1 and s-fractalkine.

Laboratory methods

Enzyme-linked immunosorbent assays (ELISAs) were used to determine serum MCP-1, TGF- β 1, and s-fractalkine concentrations. Samples were examined with commercial kits according to the manufacturers' instructions; human MCP-1, human TGF- β 1 (Biosource, Camarillo, CA), and s-fractalkine (R&D systems, Minneapolis, MN). MCP-1 and TGF- β 1 assays were run according to the protocol that was recommended in the kit. S-fractalkine was measured according to the protocol mentioned below. S-fractalkine was assessed using the basic components required by the manufacturer, and briefly, for the plate preparation, 4 μ g/mL of mouse antibody to human fractalkine was used as the capture antibody with 96 well low-cell-binding EIA plates (Nunc, Roskilde, Denmark) in an overnight incubation at 25°C.

Table 1 CP staging system (partial modification of reference 12, 16, 17)

Exocrine pancreatic function (score, 0–3)	
Each of the following abnormalities is scored as 1: Decreased serum level of pancreatic amylase or trypsin, abnormal bentiromide-para amino benzoic acid (BT-PABA) test result, and low fecal chymotrypsin.	
0	No abnormalities in the above examination
1	Total score 1 in the above examinations
2	Total score 2 in the above examinations
3	Total score 3 in the above examinations
Pancreatography by endoscopic retrograde cholangiopancreatography (ERCP; score, 0–4)	
0	Normal
1	Slightly abnormal (simple dilatation of the main pancreatic duct or localized and irregular dilation of two to three branches)
2	Mild pancreatitis (diffuse and irregular mild dilatation of the main pancreatic duct or branches, or moderate dilatation of the main pancreatic duct localized in the body and/or tail of the pancreas)
3	Moderate pancreatitis (diffuse and irregular moderate dilatation of the main pancreatic duct or branches, or advanced dilation of the main pancreatic duct localized in the body and/or tail of the pancreas)
4	Severe pancreatitis (diffuse and irregular advanced dilatation of the main pancreatic duct and branches)
Glucose metabolism (score, 0–4)	
0	Normal glucose tolerance (urine glucose negative; postprandial glucose < 160 mg/dL)
1	Slightly impaired glucose tolerance (impaired glucose tolerance after oral glucose loading test; postprandial glucose, > 160 mg/dL, < 200 mg/dL)
2	Mild diabetes mellitus (urine glucose positive after meal; postprandial glucose, > 200 mg/dL; < 300 mg/dL; HbA1c < 7%)
3	Moderate diabetes mellitus (postprandial glucose, > 300 mg/dL; HbA1c 7%–11%)
4	Severe diabetes mellitus (HbA1c > 11%, diabetic retinopathy, or diabetic nephropathy)
Pain (evaluated in the previous 1 yr, score 0–4)	
0	No or only slight pain (requires no analgesics)
1	Mild pain (occasional pain but requires no analgesics)
2	Moderate pain (frequent pain attacks, often requires analgesics)
3	Severe (always requires analgesics)
4	Most severe (requires frequent injections of analgesics, and, often, hospitalization)
Alcohol intake (score, 0–2)	
0	Less than 180 mL sake ¹ , not every day
1	Less than 540 mL sake, almost every day
2	More than 540 mL sake, almost every day
Complications associated with chronic pancreatitis (score, 0–2)	
0	No complications such as pseudocyst and stenosis of the biliary tract
1	Complications that require no treatment
2	Complications that require treatment
Total score	Severity of chronic pancreatitis
0–3	Mild
4–7	Moderate
> 8	Severe

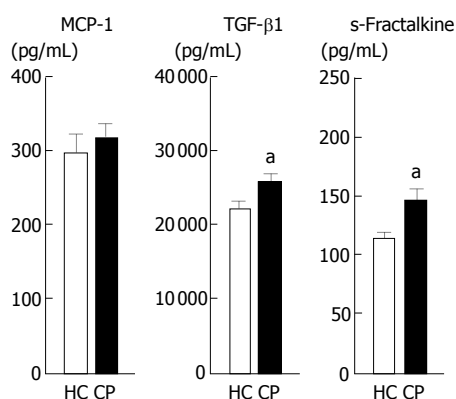
¹Sake and shochu are typical Japanese alcoholic beverages, with an ethanol content of about 16% and 25%, respectively. One unit of sake, which contains 29 g of ethanol, represents one Japanese drink.

Next, the plate was blocked with phosphate buffer saline (PBS) containing 10% fetal bovine serum (Invitrogen, Auckland, New Zealand), biotinylated mouse antibody to human fractalkine (500 μ g/mL) was added after serum samples and standard, and were incubated for two hours at 25°C. Recombinant human fractalkine (R&D systems) was used as a standard. After that, the plate was incubated with streptavidin conjugated to horseradish peroxidase (HRP) for 20 min at 25°C in the

Table 2 Number of each classification in patients with CP

Score	0	1	2	3	4	Total
Pancreatic imaging tests	0	47	33	17	12	109
Exocrine function	31	66	12	0	0	109
Glucose metabolism	63	18	13	4	11	109
Pain	65	35	7	2	0	109
Alcohol intake	57	30	22	N/A	N/A	109
Complications	90	17	2	N/A	N/A	109
Stage of severity (total score)	Mild (0-3) 36	Moderate (4-7) 49	Severe (> 8) 24	109		

N/A: Not applicable.

**Figure 1** Serum MCP-1, TGF- β 1, and s-fractalkine concentrations in patients with CP. Serum MCP-1, TGF- β 1, and s-fractalkine concentrations were measured by ELISA for each pancreatic disease. Healthy control (HC, $n = 116$) and chronic pancreatitis (CP, $n = 109$). Bars represent the mean \pm SD. ^a $P < 0.05$ vs healthy control.

dark. Finally, antibody complex was detected by base on the formation of the avidin-biotin complex, and the color was developed with tetramethylbenzidine (TMB) solution by incubating for 30 min at 25°C in the dark. Absorbance was read at 450 nm/570 nm.

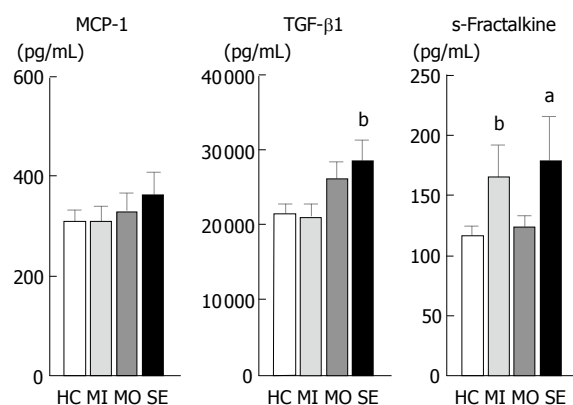
Statistical analysis

Statistical analysis was performed using the non-parametric Mann-Whitney U test. P values less than 0.05 were considered significant. Pearson's correlation analysis was used to calculate correlations between the data.

RESULTS

Serum MCP-1, TGF- β 1, and s-fractalkine concentrations in patients with CP (Figure 1)

Serum MCP-1 levels in patients with CP were not significantly elevated. On the other hand, serum TGF- β 1 levels in patients with CP were significantly higher than those of healthy controls ($P = 0.029$). Furthermore, s-fractalkine levels in CP were also significantly increased when compared to those of healthy controls ($P = 0.011$). Thus, we next analyzed whether specific grading factors as pancreatic imaging tests, exocrine function, glucose metabolism, pain, alcohol intake, or complications and severity of CP are related to serum MCP-1, TGF- β 1, and s-fractalkine concentrations.

**Figure 2** Serum MCP-1, TGF- β 1, and s-fractalkine concentrations with regard to severity of CP. Serum MCP-1, TGF- β 1, and s-fractalkine concentrations were measured by ELISA for each stage of severity. Healthy control (HC, $n = 116$), mild severity (MI, $n = 36$), moderate severity (MO, $n = 49$), and severe (SE, $n = 24$). Bars represent the mean \pm SD. ^a $P < 0.05$ vs healthy control, ^b $P < 0.01$ vs healthy control.

Serum MCP-1, TGF- β 1, and s-fractalkine concentrations in relation to the severity stage of CP (Figure 2)

First, we examined the MCP-1, TGF- β 1, and s-fractalkine levels in each stage of severity. Serum TGF- β 1 levels in the severe stage of CP were significantly higher than in healthy controls ($P = 0.008$). On the contrary, serum s-fractalkine levels in the mild and in the severe stage of CP were significantly elevated compared to healthy controls ($P = 0.004$ and $P = 0.046$, respectively). However, the serum MCP-1 level didn't significantly increase for each stage of severity.

Serum MCP-1, TGF- β 1, and s-fractalkine concentrations in relation to imaging test scores in patients with CP (Figure 3)

Serum of MCP-1, TGF- β 1, and s-fractalkine levels were analyzed regarding imaging scores. Serum TGF- β 1 levels in patients with CP revealed a score of > 3 in pancreatic imaging tests and was found to be significantly enhanced when compared to healthy controls ($P = 0.0001$). On the other hand, serum s-fractalkine levels in patients with a score of 1 and a score of ≥ 3 were significantly elevated when compared to healthy controls ($P = 0.010$ and $P = 0.041$, respectively). However, no relationship was found between serum MCP-1 levels and any level of the imaging tests.

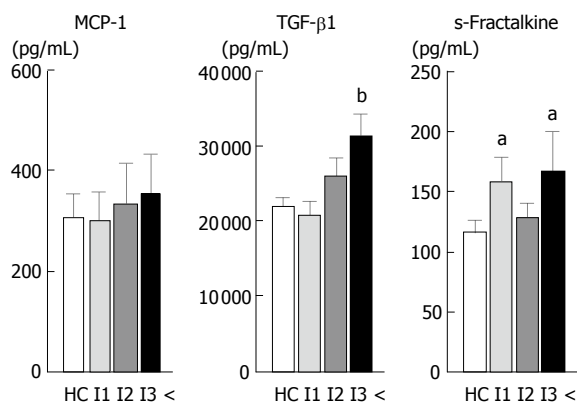


Figure 3 Serum MCP-1, TGF-β1, and s-fractalkine concentrations for each score of imaging tests in CP patients. Serum MCP-1, TGF-β1, and s-fractalkine concentrations were measured by ELISA for each score for pancreatic imaging tests. Healthy control (HC, $n = 116$), score 1 on pancreatic imaging tests (I1, $n = 47$), score 2 on pancreatic imaging tests (I2, $n = 33$) and score ≥ 3 on pancreatic imaging tests ($\geq I3$, $n = 29$). Bars represent the mean \pm SD. ^a $P < 0.05$ vs healthy control, ^b $P < 0.01$ vs healthy control.

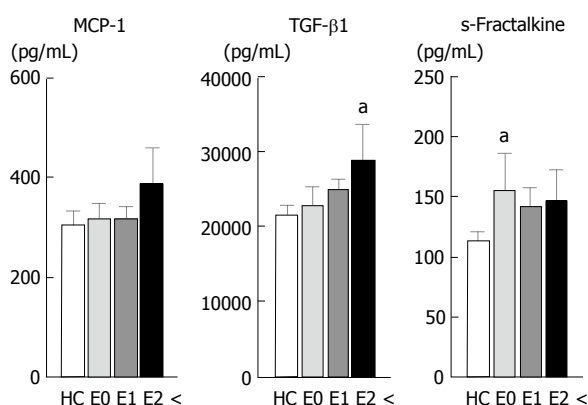


Figure 4 Serum MCP-1, TGF-β1, and s-fractalkine concentrations for each score for the exocrine function tests in CP patients. Serum MCP-1, TGF-β1, and s-fractalkine concentrations were measured by ELISA for each score for the exocrine function. Healthy control (HC, $n = 116$), score 0 for exocrine function (E0, $n = 31$), score 1 for exocrine function (E1, $n = 66$) and score ≥ 2 for exocrine function ($\geq E2$, $n = 12$). Bars represent the mean \pm SD. ^a $P < 0.05$ vs healthy control.

Serum MCP-1, TGF-β1, and s-fractalkine concentrations in relation to exocrine function test scores in patients with CP (Figure 4)

Serum TGF-β1 levels in patients with an exocrine function score ≥ 2 were significantly higher than in healthy controls ($P = 0.042$). In contrast, serum s-fractalkine was significantly elevated in patients with an exocrine function score of 0 compared to healthy controls ($P = 0.030$). However, no significant relationship was observed between serum MCP-1 levels and the exocrine function test.

Serum MCP-1, TGF-β1, and s-fractalkine concentrations for each score of alcohol intake in patients with CP (Figure 5)

Serum TGF-β1 and s-fractalkine levels in patients with an alcohol intake score of ≥ 1 were significantly higher than in healthy controls ($P = 0.018$ and $P = 0.010$,

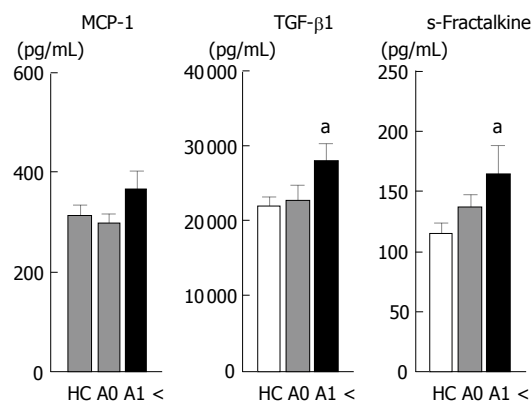


Figure 5 Serum MCP-1, TGF-β1, and s-fractalkine concentrations in relation to alcohol intake in CP patients. Serum MCP-1, TGF-β1, and s-fractalkine concentrations were measured by ELISA for each score for alcohol intake. Healthy control (HC, $n = 116$), score 0 for score of alcohol intake (A0, $n = 57$) and score ≥ 1 for score of alcohol intake ($\geq A1$, $n = 52$). Bars represent the mean \pm SD. ^a $P < 0.05$ vs healthy control.

respectively). Serum MCP-1 levels, in contrast, appeared not to be related to alcohol consumption.

Analysis of serum MCP-1, TGF-β1, and s-fractalkine concentrations on other factors in patients with CP

Finally, serum MCP-1, TGF-β1, and s-fractalkine concentrations were analyzed in relationship to other grading factors. However, we did not observe any significant relation among them. Furthermore, serum MCP-1, TGF-β1, and s-fractalkine levels did not correlate with each other. Moreover, MCP-1, TGF-β1, and s-fractalkine levels also did not correlate with age, sex, or serum pancreatic enzymes (p-amyase and lipase).

Serum TGF-β1 concentrations in non-alcoholic CP

In order to assess the influence of alcohol intake, we next focused on 57 patients with non-alcoholic CP. In the classification of severity for CP, patients with CP in the moderate stage alone showed a significant increase in serum TGF-β1 compared to healthy controls ($P = 0.039$, Figure 6A). In the classification of imaging tests and exocrine function tests, patients with CP revealing a moderate progressive stage tended to have an increase in serum TGF-β1 (Figure 6B and C).

Serum s-fractalkine concentrations in non-alcoholic CP

Similarly, we examined serum s-fractalkine in patients with non-alcoholic CP. In the classification of severity and pancreatic imaging tests, patients with CP in a mild stage alone and with a score of 1 alone showed a significant increase in serum s-fractalkine compared to healthy controls ($P = 0.026$ and $P = 0.025$, respectively; Figure 7A and B). Serum s-fractalkine didn't have a tendency for the exocrine function test (Figure 7C).

DISCUSSION

Recently, the impact of chemokines and cytokines have been recognized in the progression of chronic inflammatory diseases^[15]. However, until now, there are

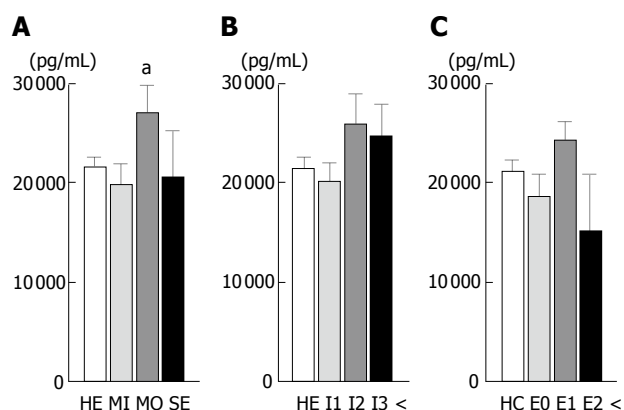


Figure 6 Serum TGF-β1 concentrations in non-alcoholic CP for each factor. Serum TGF-β1 concentrations were measured in patients with non-alcoholic CP ($n = 57$) by ELISA. A: Serum TGF-β1 concentrations for each stage of severity: Healthy control (HE, $n = 116$), mild severity (MI, $n = 33$), moderate severity (MO, $n = 19$) and severe (SE, $n = 5$); B: Serum TGF-β1 concentrations for each imaging test score: Healthy control (HE, $n = 116$), score 1 for imaging tests score (I1, $n = 33$), score 2 for imaging tests score (I2, $n = 15$) and score < 3 for imaging tests score (< I3, $n = 9$); C: Serum TGF-β1 concentrations for each score for exocrine function. Healthy control (HC, $n = 116$), score 0 for exocrine function (E0, $n = 16$), score 1 for exocrine function (E1, $n = 37$) and score > 2 for exocrine function (> E2, $n = 4$). Bars represent the mean ± SD. * $P < 0.05$ vs healthy control.

only few reports related to the serum chemokines and cytokines levels in patients with pancreatic diseases, especially CP. Therefore, in the present study, we examined serum MCP-1, TGF-β1, and s-fractalkine concentrations that are supposed to be involved in the progression of chronic inflammatory diseases in patients with CP. In patients with CP, TGF-β1 and s-fractalkine levels, but not MCP-1 levels, were significantly increased compared to healthy controls. CP patients were classified according to severity of disease using a staging system which is based on clinical symptoms and pancreatic functions^[12-14] and analyzed for the relationship with those chemokines and cytokines in order to assess the usefulness of these mediators as biological or functional markers of CP.

MCP-1, a family member of C-C chemokines, is known to play an important role in the development of the pancreatic fibrosis in CP. Previously, we reported that MCP-1 is expressed strongly in mild to moderate stages of pancreatic fibrosis in CP model rats, and suggested MCP-1 to be a pro-fibrogenic factor for CP^[16,17]. Furthermore, we showed fibrosis of CP is inhibited by blocking MCP-1^[18,19]. In the present study, we thus examined whether MCP-1 might be a useful marker in CP. MCP-1 levels in patients with CP, however, were not significantly higher than in healthy controls. Also in previous reports, serum MCP-1 didn't increase in patients with CP^[20]. Consequently, it is thought that serum MCP-1 doesn't become a useful marker in the diagnosis of CP.

TGF-β1 is a homodimeric, multifunctional cytokine^[21]. Until now, it has been generally well understood that TGF-β1 plays an important role in the development of the pancreatic fibrosis in CP^[22]. TGF-β1 is thought to be expressed in pancreatic stellate cells

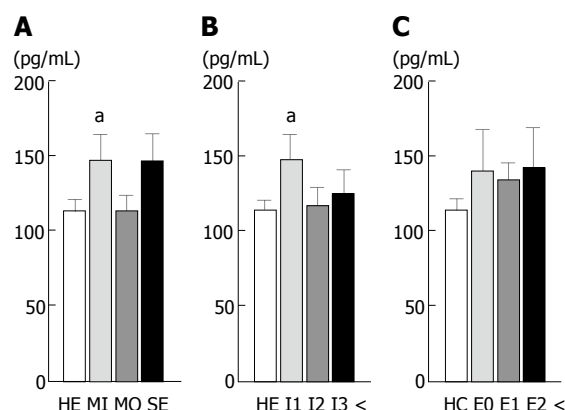


Figure 7 Serum s-fractalkine concentrations in non-alcoholic CP for each factor. Serum s-fractalkine concentrations were measured for patients with non-alcoholic CP ($n = 57$) by ELISA. A: Serum s-fractalkine concentrations were measured by ELISA for each stage of severity: Healthy control (HE, $n = 116$), mild severity (MI, $n = 33$), moderate severity (MO, $n = 19$) and severe (SE, $n = 5$); B: Serum s-fractalkine concentrations were measured by ELISA for each imaging test score: Healthy control (HE, $n = 116$), score 1 for imaging tests score (I1, $n = 33$), score 2 for imaging tests score (I2, $n = 15$), and score < 3 for imaging tests score (< I3, $n = 9$); C: Serum s-fractalkine concentrations for each score of exocrine function. Healthy control (HC, $n = 116$), score 0 for exocrine function (E0, $n = 16$), score 1 for exocrine function (E1, $n = 37$) and score > 2 for exocrine function (> E2, $n = 4$). Bars represent the mean ± SD. * $P < 0.05$ vs healthy control.

(PSC) and acinar cells closer to pancreatic fibrosis, and to regulate the synthesis of collagen from PSC^[23-25]. In our results, serum TGF-β1 levels of the patients with CP elevated significantly in the more severe stages of CP, especially in the group with more advanced scores in pancreatic imaging and exocrine function tests. These findings suggest that serum TGF-β1 levels tended to increase significantly in patients with more advanced CP. Previously, Su *et al*^[26] reported the expression of TGF-β1 in pancreatic tissue in WBN/Kob rats which is considered as a CP model, and demonstrated that expression of TGF-β1 and fibronectin showed a peak at 12 wk, whereas pancreatic fibrosis peaked at 16 wk. It was concluded that TGF-β1 may trigger fibrogenesis. Furthermore, Detlefsen *et al*^[27] classified the pancreatic tissue from CP patients into histological staging by an inflammatory process, and showed that TGF-β1 receptors had expressed predominantly in the early to moderate stage of pancreatic fibrosis. Given these data, we had expected that TGF-β1 levels might increase in the progressive process of pancreatic fibrosis for the present study. However, our results showed that TGF-β1 levels are elevated in patients with most advanced CP. On the other hand, interestingly, CP patients with high alcohol consumption showed a significant increase in levels of TGF-β1 (Figure 5). Concerning other organs, such as the liver and lungs, it is well known that the expression of TGF-β1 increases in the tissue of local organs from the effects of alcohol^[28,29]. Therefore, in the present study, in order to clarify whether serum TGF-β1 levels might increase by TGF-β1 originated from various organs under the influence of alcohol, we focused on 57 patients with non-alcoholic CP who are not influenced by alcohol, and analyzed similarly the

relationship between TGF- β 1 and each factor (Figure 6). For the classification of severity of CP, patients with CP in the moderate stage alone showed a significant increase in serum TGF- β 1 compared to healthy controls. Furthermore, in the classification of imaging tests and exocrine function tests, CP patients revealing a moderate progressive stage tended to have higher serum TGF- β 1 levels. These results are in accordance with previous reports^[26,27]. That is to say, our results support the idea that serum TGF- β 1 levels might increase because the expression of TGF- β 1 was elevated in the moderate stage in which fibrosis had been proceeding in a broad range of pancreatic tissue. On the other hand, serum TGF- β 1 levels might decrease in severe stages because pancreatic tissue had been already replaced with fibrosis. Taken together, it is suggested that the determination of serum TGF- β 1 levels might be useful to diagnose moderate stages in patients with non-alcoholic CP.

Fractalkine/CX3CL1, a family member of CX3C chemokines, has recently been reported to be expressed as a membrane-spanning adhesion molecule that can be cleaved from the cell surface to produce a soluble chemoattractant^[30-33]. The expression of fractalkine has been observed on various cells such as epithelial cells or endothelial cells of several organs. Membrane-bound fractalkine (m-fractalkine) is shed by metalloproteinase, and releases s-fractalkine^[34]. M-fractalkine functions as an adhesion molecule, whereas s-fractalkine acts as a chemoattractant and recruits inflammatory cells expressing fractalkine receptors such as monocytes^[33]. In inflamed local organs, such as the liver, lungs, and the kidneys, the participation of fractalkine has been recently noted^[35-40]. Furthermore, increased s-fractalkine serum levels have been reported for patients with various chronic inflammatory diseases^[40-44]. However, until now, there are no reports related to fractalkine in pancreatic inflammatory diseases. In the present study, we measured s-fractalkine in the serum of patients with CP. We found serum s-fractalkine levels to be significantly elevated in patients with CP. In classification of severity and pancreatic imaging tests, serum s-fractalkine levels showed significant bisferious increase in mild and severe stages. In classification of the exocrine function tests, serum s-fractalkine levels increased in mild stages alone. On the other hand, CP patients with high alcohol consumption showed a significant increase in s-fractalkine levels, similar to those of TGF- β 1 (Figure 5). Since the relationship between alcohol and fractalkine was still unclear in organs, including the pancreas, we focused on 57 patients with non-alcoholic CP, and analyzed the relationship between s-fractalkine and each factors, similar to TGF- β 1 (Figure 7). In classification of severity and pancreatic imaging tests, only patients with CP in the mild stage alone showed a significant increase in serum s-fractalkine compared to healthy controls. Thus, in patients with non-alcoholic CP, the measurement of serum s-fractalkine may be useful biological and functional markers to diagnose early-stage CP.

In conclusion, it is suggested that the measurement

of serum TGF- β 1 may be available to diagnose moderate stage of non-alcoholic CP, and that the measurement of serum s-fractalkine may be useful to diagnose early stages of non-alcoholic CP. Therefore, the measurement of a combination of TGF- β 1 and s-fractalkine may be helpful to evaluate the status of CP.

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COMMENTS

Background

Chronic pancreatitis (CP) is a chronic clinical disorder characterized by irreversible damage to the pancreas. Unfortunately, simple, indirect measurements of decreased pancreatic function have not shown abnormality until CP is advanced. The quest continues for useful biological and functional markers of early-stage CP. Recently, the roles of chemokines and cytokines have been made clear in the progression of chronic inflammatory diseases. Similarly, chemokines and cytokines have been recognized as important factors in the progression of CP. However, until now, there are only few reports addressing serum chemokine and cytokine levels in patients with pancreatic diseases, especially CP.

Research frontiers

Recently, it is widely accepted that pancreatic stellate cells are responsible for the progression of pancreatic fibrosis production of an extracellular matrix, chemokines and cytokines. Especially, the expression of transforming growth factor beta-1 (TGF- β 1) is, prior to pancreatic fibrosis in WBN/Kob rats, supposed to be a trigger for the fibrogenic process. Monocyte chemoattractant protein-1 (MCP-1) is related to the pancreatic fibrosis in di-n-butyl tin dichloride (DBTC)-induced rats. Next, it has been reported that soluble type fractalkine (s-fractalkine) increased in the serum of patients with various chronic inflammatory diseases: Atopic dermatitis, the nervous system, lupus erythematosus, rheumatoid vasculitis, and pityriasis rosea. Therefore, the purpose is to investigate whether the determination of serum MCP-1, TGF- β 1, and s-fractalkine concentration can become a useful biological and functional marker of CP using large number of CP patients classified by severity with a staging system.

Innovations and breakthroughs

Serum TGF- β 1 levels of the patients with CP tended to increase in the patients with more advanced CP, whereas serum s-fractalkine levels showed bimodal increase in mild and severe stages. However, it was observed that both TGF- β 1 and s-fractalkine levels were affected by alcohol intake. Thus, serum TGF- β 1 showed significant increase in the moderate stage of CP, and serum s-fractalkine revealed significant increase in the early stage of CP, when removed alcoholic CP. Therefore, the measurement of serum TGF- β 1 may be available to diagnose moderate stage of non-alcoholic CP, and that the measurement of serum s-fractalkine may be useful to diagnose early stages of non-alcoholic CP. The measurement of a combination of TGF- β 1 and s-fractalkine may be helpful to evaluate the severity status of CP.

Applications

Patients with CP fulfilled clinical diagnostic criteria for CP by the Japan Pancreas Society and healthy control patients excluded with recent inflammatory diseases such as infectious diseases and chronic hepatitis, and a large scale of drinking were selected. We classified the CP patients into stages of severity by the modified staging system which consists of 6 grading factors. Then, we analyzed whether each grading factor (pancreatic imaging tests, exocrine function, glucose metabolism, pain, alcohol intake, complications) and severity of CP are related with serum MCP-1, TGF- β 1 or s-fractalkine levels. Enzyme-linked immunosorbent assays (ELISA) were performed to quantify serum MCP-1, TGF- β 1, and s-fractalkine concentrations.

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The conclusion of the study is that serum s-fractalkine determination is useful at the early stage of the disease. First, the classification of severity of chronic pancreatitis seems complicated since involving 6 grading factors. Apart from the clinical course and histological stage, the staging system for CP, based on

clinical symptoms and pancreatic functions has been proposed in Japan. Next, to analyze a third group, a group of "healthy" but alcoholic controls without liver or other organic disease would be interesting although they are excluded in this study. An interesting perspective of this work should be the longitudinal evolution of this marker during progression of the disease.

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