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Role of plasma LPL and GPIHBP1 in patients with pancreatitis

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Abstract

Plasma LPL and GPIHBP1 levels and other lipid metabolic factors in patients with pancreatitis with blood triglycerides in the normal range were measured over time. The subjects included patients with acute pancreatitis (AP) (n=57) and chronic pancreatitis (CP) (n=30), and 20 healthy controls. Plasma LPL and GPIHBP1 were measured by enzyme-linked immunosorbent assay, apo protein by immunoturbidimetric assay, and other lipids by routine methods. Disease status was based on the APACHE-II score at the time of blood sampling. AP cases showed a significant decrease in LPL and GPIHBP1 compared to controls, whereas CP cases showed a non-significant decrease. ApoC2 II and III levels in patients with pancreatitis were lower than those in controls. LPL and GPIHBP1 levels were significantly positively correlated with each other, and both showed a significant negative correlation with the APACHE-II score. LPL and GPIHBP1 levels in pancreatitis survivors and non-survivors showed a trend towards a decrease with worsening disease and an increase with recovery. Serum triglyceride levels showed a non-significant negative correlation with LPL and GPIHBP1 levels. These results suggest that pancreatitis is related to abnormal lipid metabolism, mainly through a decrease in LPL in the course of the disease.

Keywords: Lipoprotein lipase; GPIHBP1; Triglyceride; Pancreatitis; Apolipoprotein; APACHE-II

1. Introduction

Pancreatitis can be a severe, recurrent and life-threatening disease, and acute pancreatitis may lead to chronic pancreatitis and diabetes. The typical causes of pancreatitis include alcohol consumption, bile stones and hypertriglyceridemia (HTG), and patients with severe HTG should be carefully monitored for possible complications of pancreatitis. Regardless of the underlying etiology of HTG, the risk of acute pancreatitis increases as triglyceride (TG) levels increase, particularly when this level exceeds 1000-2000 mg/dl. Clinical features of HTG-related pancreatitis do not differ from those of pancreatitis of other etiologies [1].

Lipoprotein lipase (LPL) is thought to be an important cause of HTG-related pancreatitis, since LPL has a central role in plasma lipid metabolism of hydrolyzing TGs in lipoproteins and releasing vital fatty acid nutrients for tissues. However, there is no clear way to identify which patients with severe HTG will develop pancreatitis [1,2]. The underlying pathophysiological concepts include hydrolysis of TG by pancreatic lipase and excessive formation of free fatty acids (FFAs) with inflammatory changes and capillary injury. Hyperviscosity and ischemia may also play decisive roles [3].

LPL is produced by parenchymal cells of peripheral organs, secreted, and anchored to heparan sulfate proteoglycans on the cell surface. Glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) transports LPL from the cell surface to the capillary endothelium [4, 5]. LPL transported to the vascular endothelium hydrolyzes TGs from chylomicrons (CMs) and very-low-density lipoprotein cholesterol in the circulation, producing FFAs and glycerol. Insulin induces LPL expression and activity, which leads to FFA uptake into peripheral organs for

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energy storage and consumption [5]. Therefore, LPL is a key regulator of TG levels that is needed for peripheral organs to store and consume lipids.

Familial CM syndrome is a rare genetic disorder estimated to affect 1 to 2 individuals per million and characterized by HTG. This disease is caused by mutations in LPL or genes that regulate LPL function, which include apoprotein (Apo) C2, ApoA5, GPIHBP1, and lipase maturation factor 1 (LMF1) [6]. The activity and stability of LPL are regulated by proteins such as angiopoietin-like proteins (ANGPTLs) and Apo proteins. Loss-of-function mutations in ApoC2 and ApoA5 inhibit LPL, resulting in severe HTG [1,2]. In contrast, ANGPTL3, 4 and 8, and ApoC3 inhibit LPL activity, thereby increasing serum TG levels [5-8] .

There have been many reports of pancreatitis associated with abnormalities of lipid metabolism, such as HTG and hyperchylomicronemia, but few studies on changes in lipid metabolism in pancreatitis [1,2]. Therefore, our aim was to examine variations in plasma levels of LPL, GPIHBP1 and other lipid metabolic factors in patients with pancreatitis with serum TGs in the normal range, at different stages of severity. The role of lipid metabolism in the disease was then investigated by examining the trends in fluctuation of these levels.

2. Material and methods

2.1. Subjects

The subjects were 57 patients with acute pancreatitis (AP); 30 patients with chronic pancreatitis (CP) and 20 age-matched healthy controls. AP consists of 14 patients with severe acute pancreatitis (SAP) and 43 patients with mild acute pancreatitis (MAP) including 5 patients with pancreas carcinoma. The patients with AP, CP and pancreas carcinoma had an average age of 52.4±12.6 years. The baseline characteristics of the patients are shown in Table 1. Diagnosis of pancreatitis was based on abdominal signs associated with high pancreatic enzymes and morphological abnormalities consistent with AP on contrast-enhanced computed tomography and ultrasonography carried out within 24 h of admission. Diagnoses of CP and pancreatic cancer were further based on ERCP and measurements of tumor markers (particularly CA19-9), respectively.

Table 1 Clinical background of patients with pancreatitis

Item	**Severe AP**		**Severe AP**	**Mild AP**	**CP**
	Non-survivors	**Survivors**			
Female	0	2	2	5	5
Male	5	7	12	38	25
Alcohol intake	4	3	7	12	18
Bile stone	1	0	1	12	0
Idiopathic	0	6	6	15	12
pancreas ca.	0	0	0	5	0
S-amylase (u/L)	12564±7532	11785±7765	12098±88754	66754±4893	3592±1749
Lipase (u/L)	4385±3317	4237±3097	4190±3423	876±438	388±289
WBC (/μ l)	18445±7875	17648±9877	18099±7916	10976±6684	8673±5971
Platelets (10^4/μl)	15.9±9,7	16.1±7.7	15.3±6.3	18.7±6.0	18.8±7.1
Blood Suger (mg/dl)	103.3±25.3	100.1±26.8	102.7±34.1	99.2±36.8	89.8±34.2
Triglyceride (mg/dl)	149.5±33.0	132.0±27.9	143.9±38.2	125.5±35.2	115.1±28.7
Apo C II(mg/dl)	2.88±0.87*	3.11±1.02**	3.01±0.83*	3.08±1.45*	3.31±1.66*
Apo CIII(mg/dl)	5.98±1.64**	6.01±1.12**	6.03±0.83**	6.25±1.15**	6.67±3.72**
APACHE-II	22～6	25～2	25～2	15～ 1	7～0

Acute Pancreatitis (AP), Chronic Pancreatitis (CP), * p<0.05 , ** p<0.01 vs Normal Control

The severity of AP was assessed using Ranson's criteria [9], APACHE-II criteria, JSS score, criteria for Intractable Disease of the Pancreas issued by the Japanese Ministry of Health, Labour and Welfare, and the criteria of the Revised Atlanta Classification [10-12]. MOF was assessed using APACHE-II [12] criteria at the time of blood sampling. Of the 14 patients with SAP, 5 died due to sepsis and MOF, and there were 9 survivors. There were also 7 shock cases (non-survival SAP 4, survival SAP 3).

2.2. Measurements of LPL, GPIHBP1 and Apoprotein

Measurements of LPL and GPIHBP1 were performed using human LPL [13] and human GPIHBP1 [14] sandwich ELISA kits (both from Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan). Post-heparin LPL was measured after intravenous injection of heparin (0.1 mg/kg) and collection of blood plasma 10 minutes later. Apo CII and Apo CIII were measured using an immunodiffusion kit (Daiichi Pharmacy. Tokyo Japan). The intra- and inter-assay coefficients of variation for the measurements were 8.6-11.4%.

2.3. Determination of white blood cell counts, platelet counts, and pancreatic enzymes

Serum and urine amylase, lipase, white blood cell counts, and platelet counts in patients and controls were measured by routine laboratory procedures. Venous blood samples were taken on admission (days 0-3: within 72 h of pain onset) and 7 and ≤14 days after admission. A total of 107 blood samples were collected in patients with AP and CP. Plasma samples were collected in 3.8% citric acid (1/10) and frozen immediately for storage at -75 ℃ until analysis.

2.4. Statistical analysis

Values are expressed as mean ± standard deviation (SD). An unpaired Student t-test was used for comparison of mean values, with $P<0.05$ considered significant.

3. Results

3.1. Plasma LPL and GPIHBP1 levels in patients with pancreatitis

The mean plasma LPL levels were 18.7±19.4 ng/ml in AP cases and 17.4±11.3 ng/ml in patients with pancreatic cancer, with both groups showing a significant decrease compared to controls. The plasma LPL level in CP cases of 29.4 ± 34.4 ng/ml was also lower than that in controls, but with no significant difference. Post-heparin LPL in a small number of patients was significantly reduced in both AP and CP compared with controls. Post-heparin LPL was about 5 times higher than pre-heparin LPL in AP cases, and about 7 times higher in CP cases (Figure 1A). Regarding GPIHBP1 levels, AP cases had a significant decrease compared to controls, whereas CP and pancreatic cancer cases showed a decrease, but without a significant difference compared to controls (Figure 1B).

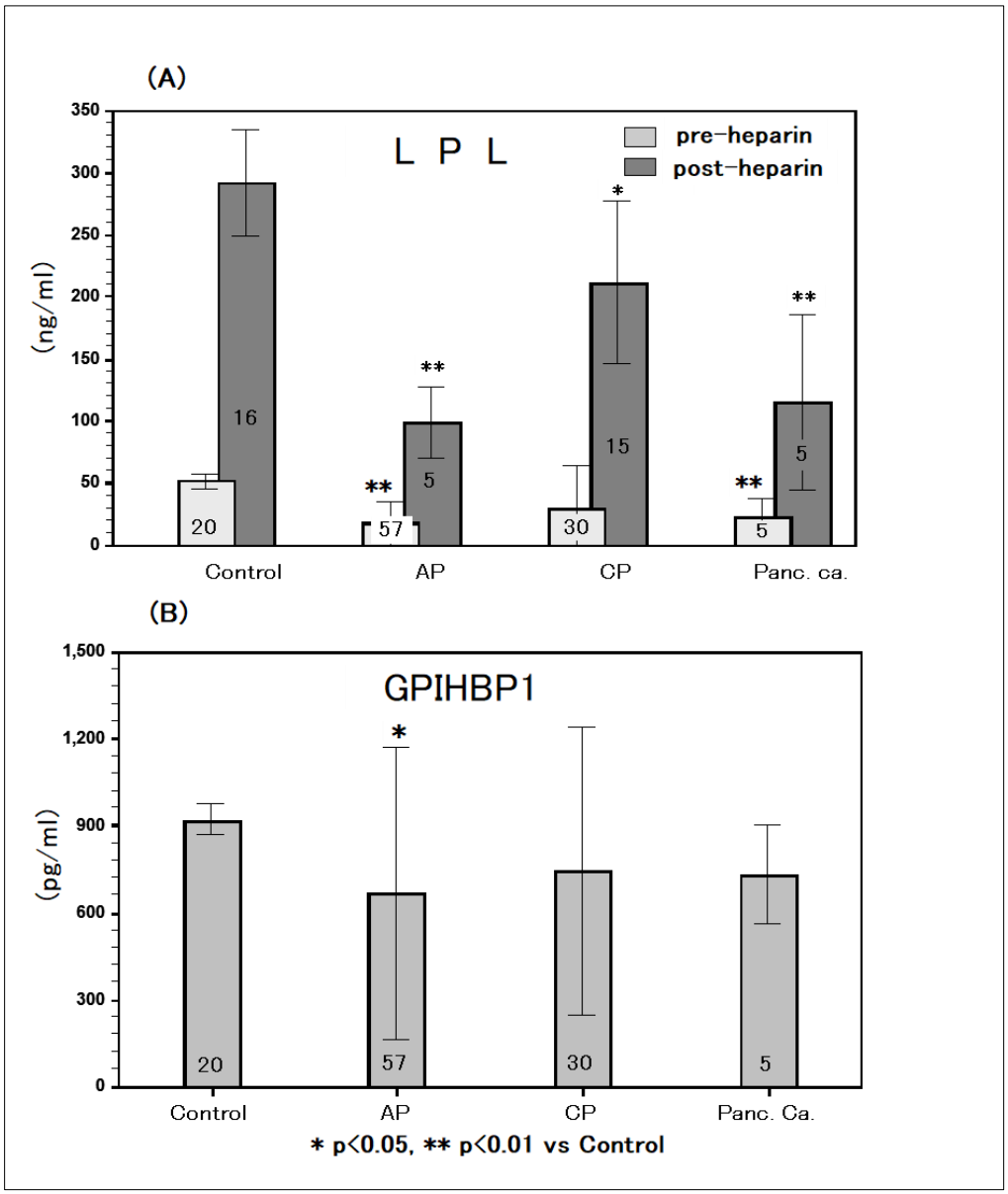

Figure 1 Plasma levels of (A) LPL pre- and post-heparin and (B) GPIHBP1 in controls and patients with acute pancreatitis (AP), chronic pancreatitis (CP), and pancreatic carcinoma (Panc. Ca)

3.2. Plasma levels of LPL and GPIHBP1 in AP survivors and non-survivors

Plasma LPL levels in survivors among AP cases were significantly higher 2 weeks after onset of AP than at disease onset. In contrast, non-survivors showed an increasing trend from onset to 1 week after onset of AP, and then a decrease at 2 weeks after onset (Figure 2A). Plasma GPIHBP1 levels in survivors gradually increased from disease onset to 2 weeks after onset, whereas those in non-survivors increased at 1 week after onset, but then decreased over the next 2 weeks (Figure 2B).

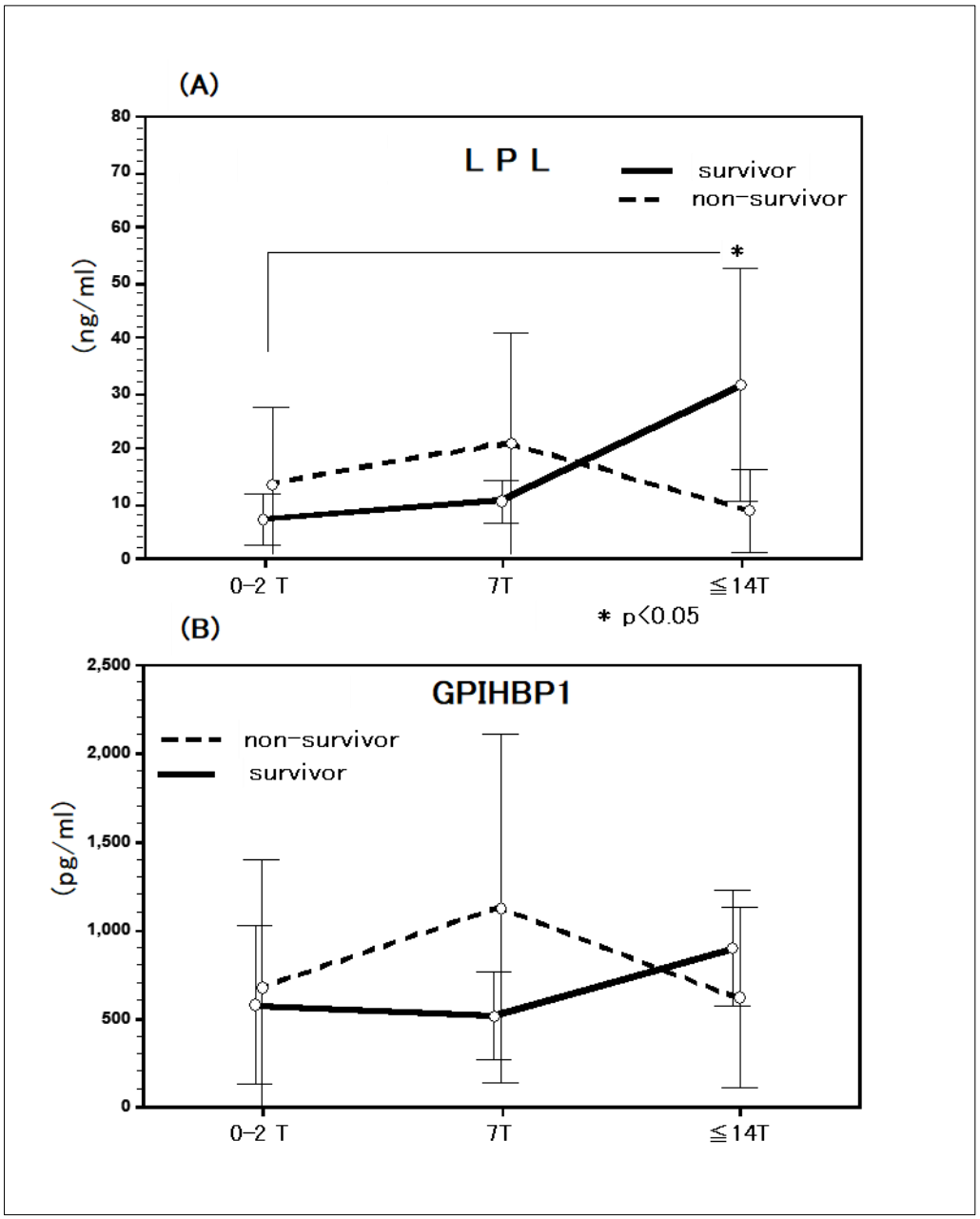

Figure 2 Changes and fluctuations in plasma levels of (A) LPL and (B) GPIHBP1 in survivors and non-survivors among patients with acute pancreatitis

3.3. Plasma LPL and GPIHBP1 levels by cause of pancreatitis

LPL levels were significantly lower in patients with pancreatitis caused by alcohol intake, bile stones and an idiopathic cause compared to controls, except for pancreatic cancer cases (Figure 3A). GPIHBP1 levels were significantly lower in AP cases with an idiopathic cause, in CP cases caused by alcohol intake, and in pancreatic cancer cases (Figure 3B).

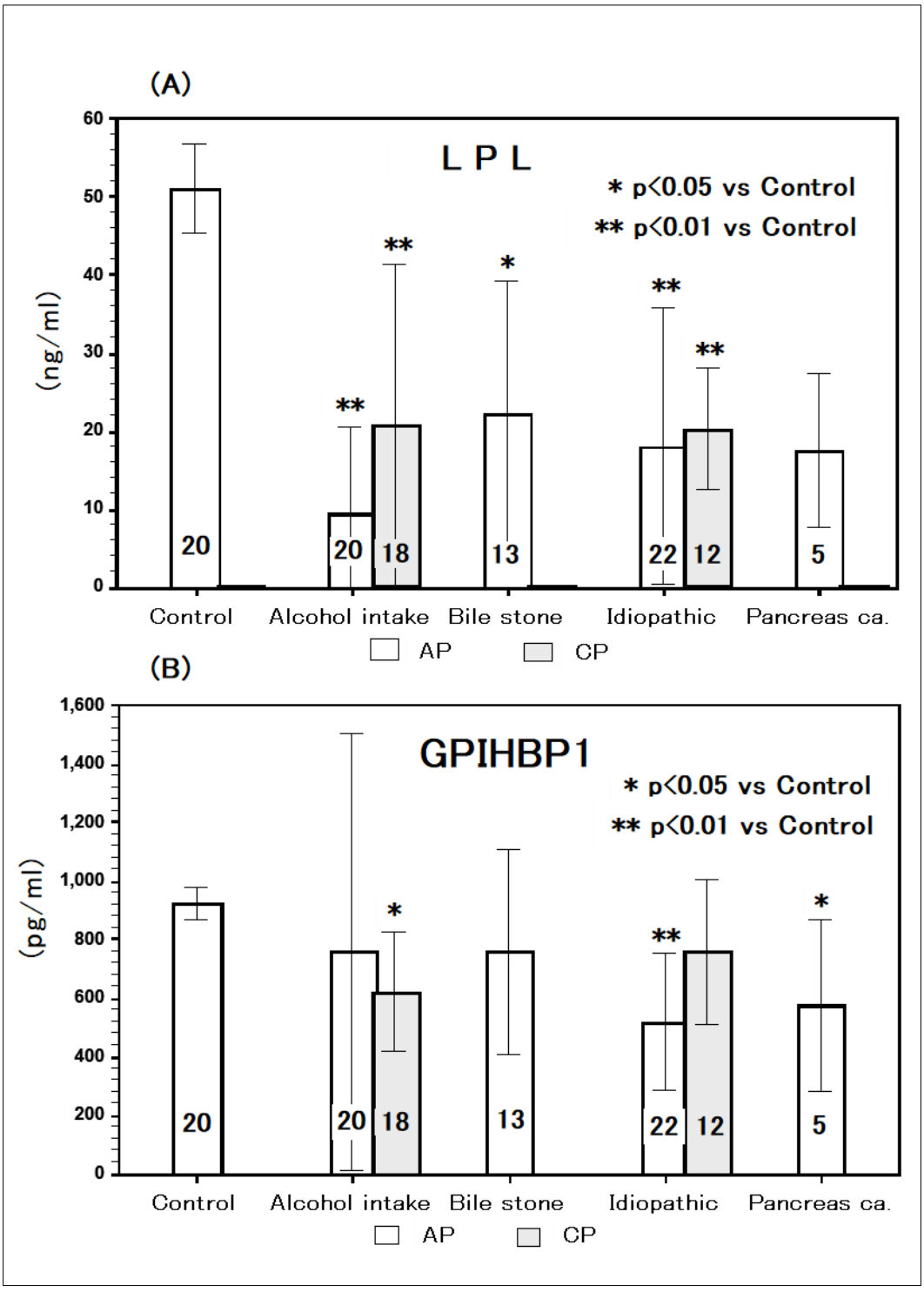

Figure 3 Plasma levels of (A) LPL and (B) GPIHBP1 by causes of disease in patients with pancreatitis

3.4. LPL/GPIHBP1 ratio and correlations with triglycerides in pancreatitis

The LPL/GPIHBP1 ratio was significantly lower in AP, CP, and pancreatic cancer cases compared to controls (Figure 4A). There were non-significant negative correlations of plasma LPL with TG (Figure 4B) and GPIHBP1 with TG (Figure 4B). There were also significant positive correlations of LPL with FFA (r=0.464) and fasting blood glucose (r=0.304).

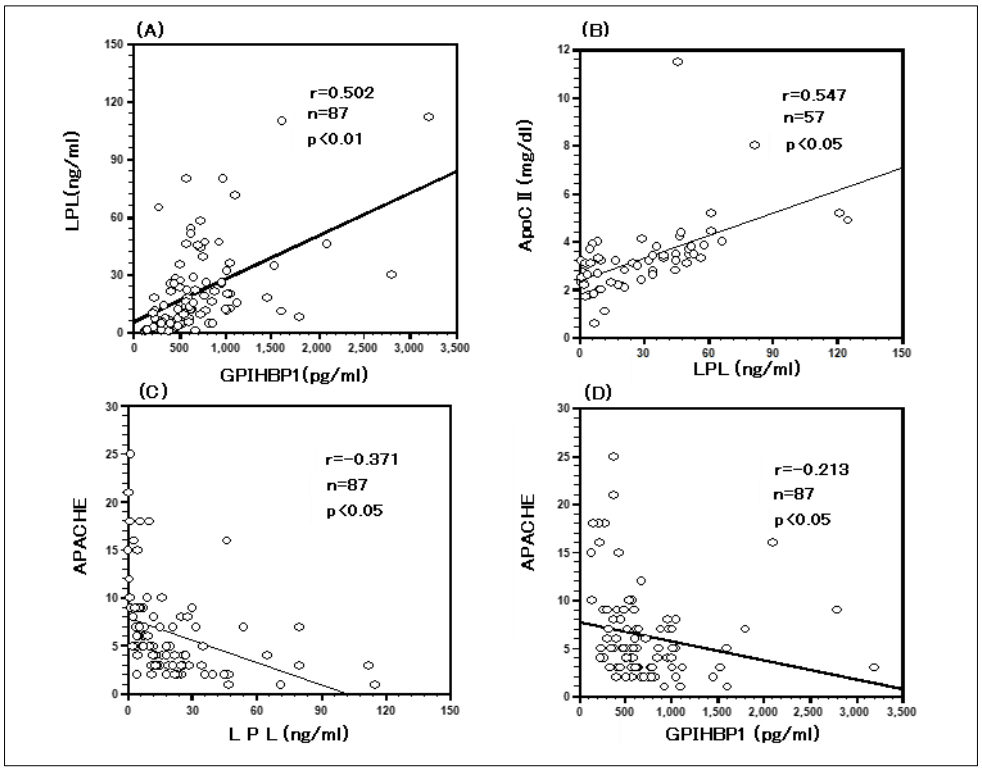

Figure 4 Correlations of (A) plasma LPL and GPIHBP1 levels, (B) ApoCII and LPL, (C) APACHE-II and LPL, and (D) APACHE-II and GPIHBP1 in patients with pancreatitis

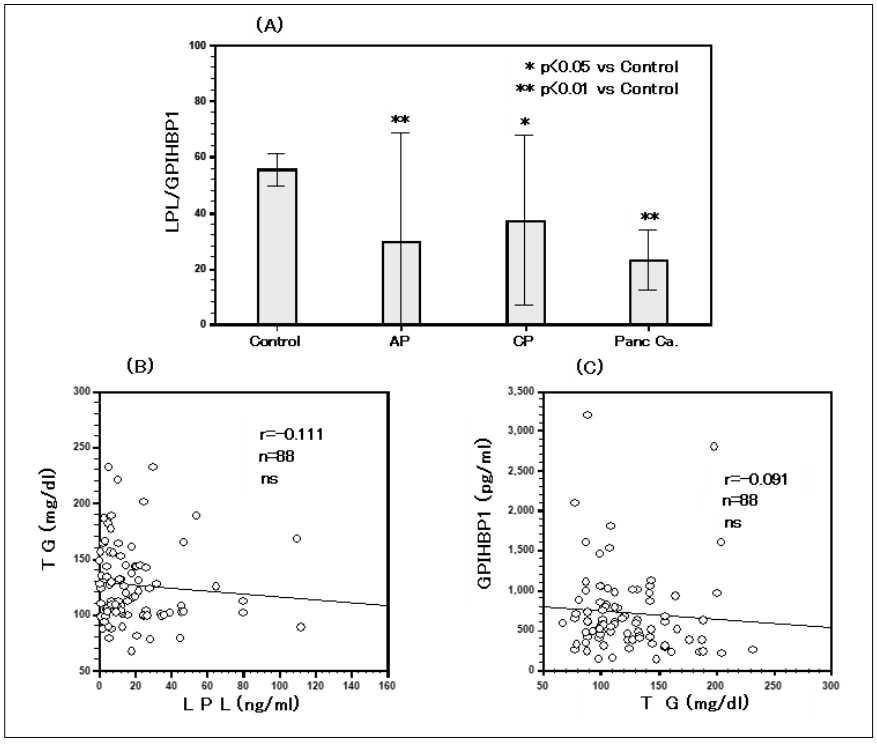

Figure 5 (A) LPL/GPIHBP1 ratio in controls and patients with acute pancreatitis (AP), chronic pancreatitis (CP), and pancreatic carcinoma (Panc. Ca). (B, C) Correlations of plasma levels of TG with those of (B) LPL and (C) GPIHBP1 in patients with pancreatitis

Patients with pancreatitis had significant positive correlations of LPL and GPIHBP1 levels (Figure 5A) and LPL and Apo C-II (Figure 5B), and significant negative correlations of APACHE-II scores with plasma levels of LPL (Figure 5C) and GPIHBP1 (Figure 5D).

4. Discussion

The role of LPL in plasma TG metabolism has been recognized for decades, but the discovery of GPIHBP1 has substantially changed textbook descriptions of intravascular triglyceride rich lipoprotein (TGRL) processing. The results of this study show that plasma LPL and GPIHBP1 levels are both reduced in patients with pancreatitis, with the reduction in LPL being greater than that in GPIHBP1. LPL and GPIHBP1 had weak negative correlations with TGs. These findings suggest the following mechanisms of LPL suppression in the blood in pancreatitis: reduced generation of LPL and GPIHBP1; reduced apolipoprotein CII, III, and V.; suppression by ANGPTL3, 4 and 8; and appearance of GPIHBP1 autoantibodies [3,15,16]. The details of these four steps may be as follows.

First, synthesis of LPL in parenchymal cells and dimerization and maturation of LPL in the endoplasmic reticulum requires LMF1. In this process, GPIHBP1 promotes LPL maturation, while ANGPTL4 is inactivated. It is of note that genetic defects in LMF1 have been reported to result in severe pancreatitis [17].

Second, the generated LPL is trapped on the interstitial side of capillary endothelial cells by GPIHBP1 generated in these and other cells, and moves to the capillary lumen; that is, LPL undergo transcytosis in capillary cells due to GPIHBP1 [18]. Immunohistochemical results show that GPIHBP1 is expressed solely in capillary endothelial cells and is absent in endothelial cells of venules, arterioles, and larger blood vessels. Binding of LPL to GPIHBP1 is mediated by the acidic and Lys6 domains of GPIHBP1, and the complexes are tethered to the vascular endothelial surface [18, 19]. GPIHBP1 is found in capillaries of all peripheral tissues, with particularly high levels in the heart and adipose tissue (where LPL-mediated processing of lipoproteins is robust), but is absent from brain capillaries. High levels of GPIHBP1 are found in lung capillaries, where LPL expression is low, but the physiologic importance of GPIHBP1 expression in the lung is unclear because a complete deficiency of GPIHBP1 does not elicit overt pulmonary abnormalities. These facts are understood as defensive reactions in response to the severity of pancreatitis [18-20].

Third, GPIHBP1 bound to LPL binds to triglycerides in the blood via ApoAV in its acidic domain and TGRL is degraded; that is, GPIHBP1 bridges TGRL and LPL. In this process, Apo CIII inhibits degradation of TGRL, while Apo CII promotes the action of TGRL and is required for maximal rates of TGRL lipolysis [21,22]. Degradation of TGRL is promoted or inhibited by ANGPTL3, 4 and 8, which consequently modulates the action of LPL [23-25]. Genetic variants of the LPL inhibiting factors (ApoAV, ApoCIII, ANGPTL3, 4) in these stages associated with increased plasma TG levels increase the risk of acute pancreatitis [26, 27]. However, these variants are rare and are associated with severe disease. Recently, Jung et al. [28] showed that ANGPTL4 accelerates the pathological exacerbation of acute pancreatitis by inducing alveolar cell damage and releasing large amounts of inflammatory cytokines. In addition, ANGPTL4 expression is elevated in the serum and pancreatic tissues of patients with pancreatitis. An effect of ANGPTL proteins in the blood on LPL is possible, but these proteins are derived from liver, fat, muscle, intestinal tract and other tissues and exert effects in combination with each other, making it unlikely that they also act in isolation. In addition, concomitant hepatic and gastrointestinal disorders are present in pancreatitis, so it is assumed that these proteins affect LPL through the totality of their mechanisms. Therefore, the effects of ANGPTLs on LPL may not necessarily be limited to inhibitory or promotive actions.

Finally, there is onset of autoantibodies that regulate LPL, GPIHBP1. LMF1, apoC-II, and apoA-V. Recently, Zhang et al. found GPIHBP1 autoantibodies in 14.7% of patients (17/116) with HTG-AP, with a 2-year recurrence rate of HTG-AP of 35% in cases with GPIHBP1 autoantibodies [29]. The decrease in LPL and GPHBP1 upon development of pancreatitis may induce impaired lipid metabolism around the capillary wall, resulting in impaired blood flow and a tendency for microthrombus formation, which may lead to ischemia in surrounding organs and cause organ damage. Thus, there is a need to understand why GPIHBP1 is expressed in capillaries, but not in larger blood vessels, and to examine correlations of TG with function-promoting and -suppressing proteins at each stage of the actions of LPL and GPIHBP1 to treat these problems. Studies are also needed to understand the initial and fluctuating levels of LPL, GPIHBP1, and other factors in pancreatitis.

5. Conclusion

In pancreatitis with a normal TG level, plasma LPL and GPHBP1 decreased at disease onset, and increased with improvement or decreased with deterioration of disease, corresponding to a decrease in TG levels. Plasma LPL and

GPHBP1 were also significantly positively correlated. From these results, we infer that other LPL suppressors fluctuated in parallel with the disease state, reflecting the tissues on which they act.

Compliance with ethical standards

Acknowledgments

We would like to thank Teijin Co., Ltd for providing ELISA kits.

Disclosure of conflict of interest

None of the authors have a conflict of interest.

Statement of ethical approval

The study was approved by the ethics committee of Hijirigaoka Hospital and performed in compliance with the Treaty of Helsinki. All patients with pancreatitis admitted to Hijirigaoka Hospital from April 2017 to February 2023 were included in the primary analysis.

Statement of informed consent

All participants gave signed informed consent before enrollment.

Author Contributions

Concept, design, and supervision: S.U, TM.; Resources, materials, data collection and processing: Y.F, T.M.; Analysis and interpretation: K.G.; Literature search and manuscript writing: S.U, TM.; Critical review: K.G.

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