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ORIGINAL ARTICLE

The Relationship between Trypsin/Calcitonin Gene Related Peptide (CGRP) in Serum and Acute Pancreatitis (AP)

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SUMMARY

Background: **Activation of trypsin from proteolytic cleavage of trypsinogen in the pancreas can lead to acute pancreatitis. Calcitonin gene related peptide (CGRP) from both peripheral and central neurons is involved in a variety of physiological/pathophysiological processes, especially sensory (nociceptive) and efferent (effector) functions. To better understand the change of trypsin/CGRP in acute pancreatitis, the study investigated the serum level of trypsin/CGRP in patients with acute pancreatitis.**
Methods: **The study investigated 140 patients with acute pancreatitis, including 72 cases of biliary acute pancreatitis, 60 cases of hyperlipidemic acute pancreatitis, and 8 cases of idiopathic acute pancreatitis. Sixty volunteers acted as the normal control group. The levels of trypsin and CGRP in serum were analyzed.**
Results: **The serum levels of trypsin and CGRP in males with acute pancreatitis were higher than in females, but there was no statistical difference ($p > 0.05$). However, the serum levels of trypsin and CGRP in different types of acute pancreatitis were significantly higher than controls ($p < 0.001$), and the level of trypsin and CGRP in serum of patients with inflammation effusion was significantly higher than patients without inflammation effusion ($p < 0.001$). In addition, the serum levels of trypsin and CGRP in patients with I-II, III, IVA and IVB acute pancreatitis were higher than controls ($p < 0.001$).**
Conclusions: **According to the results, we concluded that the trypsin and CGRP in serum can act as a new detection index of acute pancreatitis occurring. The serum levels of trypsin and CGRP in patients with acute pancreatitis is able to determine whether inflammation effusion happens.**
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INTRODUCTION

Acute pancreatitis (AP), a sudden inflammation of the pancreas, is believed to be a premature activation of digestive enzymes within the pancreatic acinar cells which is a crucial initiating event resulting in autodigestion of the pancreas [1-3]. Acute pancreatitis involves a complex cascade of events, which starts in the pancreatic acinar cells, and induces an injury or disruption of the pancreatic acini, which allows the leakage of pan-

creatic enzymes (namely trypsin) breaking down pancreatic tissue and cell membranes, causing edema, vascular damage, hemorrhage, and necrosis [4]. However, the activity of trypsin is properly inhibited in the pancreatic acinar cells under normal physiological conditions. A small amount of trypsinogen is only converted to active trypsin and inactivated by pancreatic secretory trypsin inhibitor (PSTI), which normally prevents the destruction of pancreatic acinar cells [5].

Calcitonin gene related peptide (CGRP), a 37-amino acid multifunctional neuropeptide [6], is produced in both peripheral and central neurons [7]. CGRP involves in a variety of physiological/pathophysiological processes. For example, CGRP induces vasodilation by the release of nitric oxide [8], mediates an inflammation response by increasing of IL-1β and IL-6 expression [9] and modulates nociceptive input [10,11]. It is noteworthy that CGRP can influence peripheral sensitization and inflammation [10] and induce the basal secretion of inflammatory cytokines in mononuclear cells isolated from human peripheral blood, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNFα) [12]. A number of inflammatory mediators are produced locally and systemically during acute pancreatitis. IL-1 and TNF have the ability to induce nearly all of the other mediators while feeding back to produce a direct noxious effect within the pancreas itself [13]. The research revealed that CGRP partially mediated the nociception in acute experimental pancreatitis [14].

Thus, in consideration of the pathogenesis and clinical characteristics of acute pancreatitis and the characteristics of the proinflammatory response of CGRP, the association between acute pancreatitis and trypsin/CGRP in serum should be deeply taken into consideration in the clinical perspective. Therefore, in order to better evaluate the relationship between the serum levels of trypsin/CGRP and acute pancreatitis in clinical perspective, 140 patients with acute pancreatitis were analyzed and studied.

MATERIALS AND METHODS

Patients

One hundred and forty patients with acute pancreatitis between January 2013 and August 2015 were included in the prospective study. All patients with acute pancreatitis in the study were from the First Affiliated Hospital of Fujian Medical University and included 72 cases of biliary acute pancreatitis, 60 cases of hyperlipidemic acute pancreatitis, and 8 cases of idiopathic acute pancreatitis. Fifty healthy volunteers acted as normal control group. The sera of all patients at different periods of the inflammation were collected and analyzed. All sera were stored at -80°C, when not in use. The case-control method was used for statistical analysis in the study.

Preparation of blood sample

Venous blood (5 mL) was collected in a vacutainer without additive, allowed to clot for 30 minutes at room temperature, and centrifuged at 3,000 x g for 5 minutes to separate serum. The serum aliquots were stored at -80°C until the analysis date. Hemolyzed samples were excluded.

Biochemical measurements

Trypsin in serum was detected by trypsin activity assay kit from AmyJet Scientific Inc. (China), and CGRP in serum was determined by human calcitonin gene related peptide (CGRP) ELISA kit from Shanghai Ke Shun Biological Technology Co., Ltd. (China).

Statistical analysis

The statistical analyses in the study were carried out by GraphPad Prism 6. The comparison of enumeration data between groups was by Chi-square test (χ^2-test), and the comparison of quantitative data between groups was by *t*-test. Values for $p < 0.05$ were considered statistically significant.

RESULTS

Comparative analysis of the general clinical data of patients with different types of acute pancreatitis

Table 1 showed the statistical data and clinical characteristics of the patients with different types of acute pancreatitis in the present research. The average ages of patients with biliary, hyperlipidemic and idiopathic acute pancreatitis were 35.26 ± 5.36, 39.66 ± 2.15, and 33.21 ± 3.32 years, respectively. Inpatient days of three different types of acute pancreatitis were 18.20 ± 10.78, 20.57 ± 11.12, and 10.08 ± 6.54 days, respectively.

Comparative analysis of the serum level of trypsin in patients with different types of acute pancreatitis

The comparisons of serum trypsin between different groups were shown in Table 2. The serum concentration of trypsin of the male patients was 103.13 ± 33.74 ng/mL and higher than that of the female patients (94.86 ± 32.51 ng/mL). The serum level of trypsin of the patients with inflammation effusion was 128.26 ± 41.65 ng/mL and obviously higher than that of the patients without inflammation effusion (74.26 ± 19.11 ng/mL). The serum levels of trypsin of biliary, hyperlipidemic, and idiopathic acute pancreatitis were 106.43 ± 14.89, 100.45 ± 21.85, and 94.20 ± 21.14 ng/mL, respectively, and distinctly higher than that of the controls (35.12 ± 10.30 ng/mL). The serum trypsin concentrations of acute pancreatitis at different grades (I-II, III, IVA and IVB) were 65.36 ± 19.88, 71.29 ± 36.41, 92.22 ± 35.13, and 124.17 ± 20.89 ng/mL, respectively.

Table 1. The comparison of the general clinical data of patients with different types of acute pancreatitis.

	Controls group (n = 50)	Biliary acute pancreatitis (n = 72)	Hyperlipidemic acute pancreatitis (n = 60)	Idiopathic acute pancreatitis (n = 8)
Age (years)	34.53 ± 4.72	35.26 ± 5.36	39.66 ± 2.15	33.21 ± 3.32
Male	25	52	38	4
Female	25	20	22	4
Inpatient days	-	18.20 ± 10.78	20.57 ± 11.12	10.08 ± 6.54

Table 2. The comparison of the serum level of trypsin in patients with different types of acute pancreatitis.

Clinicopathologic information		Serum level of trypsin (ng/mL)	p
Gender	Male (n = 94)	103.13 ± 33.74	0.0852
	Female (n = 46)	94.86 ± 32.51	
Inflammation effusion	Yes (n = 34)	128.26 ± 41.65	< 0.001
	No (n = 106)	74.26 ± 19.11	
Types of acute pancreatitis	Biliary (n = 72)	106.43 ± 14.89	< 0.001
	Hyperlipidemic (n = 60)	100.45 ± 21.85	< 0.001
	Idiopathic (n = 8)	94.20 ± 21.14	< 0.001
	Controls (n = 50)	35.12 ± 10.30	
Grade of acute pancreatitis	I - II (n = 46)	65.36 ± 19.88	< 0.001
	III (n = 59)	71.29 ± 36.41	< 0.001
	IVA (n = 27)	92.22 ± 35.13	< 0.001
	IVB (n = 8)	124.17 ± 20.89	< 0.001

Table 3. The comparison of the serum level of CGRP in patients with different types of acute pancreatitis.

Clinicopathologic information		Serum level of CGRP (pg/mL)	p
Gender	Male (n = 94)	91.23 ± 36.91	0.0527
	Female (n = 46)	80.96 ± 30.74	
Inflammation effusion	Yes (n = 34)	125.32 ± 54.18	< 0.001
	No (n = 106)	65.23 ± 32.14	
Types of acute pancreatitis	Biliary (n = 72)	105.15 ± 25.16	< 0.001
	Hyperlipidemic (n = 60)	95.49 ± 28.54	< 0.001
	Idiopathic (n = 8)	124.14 ± 37.53	< 0.001
	Controls (n = 50)	43.68 ± 11.65	
Grade of acute pancreatitis	I - II (n = 46)	67.52 ± 24.77	< 0.001
	III (n = 59)	86.14 ± 15.84	< 0.001
	IVA (n = 27)	99.45 ± 22.61	< 0.001
	IVB (n = 8)	129.66 ± 36.29	< 0.001

Comparative analysis of the serum level of CGRP in patients with different types of acute pancreatitis

Table 3 showed the difference of CGRP between different groups. The serum level of CGRP of the male patients was 91.23 ± 36.91 pg/mL and higher than that of the female patients (80.96 ± 30.74 pg/mL). The serum concentration of CGRP of the patients with inflammation effusion was 125.32 ± 54.18 pg/mL and significantly higher than that of the patients without inflammation effusion (65.23 ± 32.14 pg/mL). The serum levels of CGRP of biliary, hyperlipidemic, and idiopathic acute pancreatitis were 105.15 ± 25.16, 95.49 ± 28.54, and 124.14 ± 37.53 pg/mL, respectively, and significantly higher than that of the controls (43.68 ± 11.65 pg/mL). The serum concentrations of CGRP of acute pancreatitis at different grades (I-II, III, IVA and IVB) were 67.52 ± 24.77, 86.14 ± 15.84, 99.45 ± 22.61, and 129.66 ± 36.29 pg/mL, respectively.

DISCUSSION

Acute pancreatitis is a common clinical condition. Acute pancreatitis can have severe complications such as fever, tachycardia, hypovolemia, tachypnea, and hypoxia with as many as 12% presenting with respiratory insufficiency, shock, or even multisystem organ failure (MSOF) [13]. Acute pancreatitis has many distinct etiologies, but other relative factors need to be taken into account. So, trypsin/CGRP and the correlation with acute pancreatitis should be analyzed in clinical practice.

Trypsin, a serine protease belonging to the chymotrypsin family is formed in the small intestine when its proenzyme form, the trypsinogen produced by the pancreas, is activated in the small intestine [15]. Activation of trypsin from proteolytic cleavage of trypsinogen in the pancreas can lead to a series of events that cause pancreatic self-digestion, resulting in pancreatitis. CGRP is primarily localized to C and Aδ sensory fibers which display a wide innervation throughout the body and have a dual role in sensory (nociceptive) and efferent (effector) function [16]. CGRP and its receptors play an important role in the regulation of peripheral vascular tone and regional organ blood flows under normal physiological and pathophysiological conditions [17]. Liu et al. reported that CGRP mediated pancreatic hyperalgesia in chronic pancreatitis [18]. Wang et al. evaluated serum levels of CGRP and substance P (SP) in patients with diabetes mellitus and coronary artery disease, showing that CGRP and SP may have a role in the pathogenesis of coronary artery disease in patients with diabetes [19]. In addition, Wick et al. reported that nociception in the L-arginine model of acute pancreatitis is partially mediated by the release of CGRP in the dorsal horn of the spinal cord [20]. Thus, we investigated the levels of trypsin and CGRP in patients with different types of acute pancreatitis.

Table 2 showed that the serum levels of trypsin in males with acute pancreatitis were higher than in females, but the differences did not achieve statistical significance ($p > 0.05$). In addition, the level of trypsin in serum of patients with inflammation effusion was significantly higher than patients without inflammation effusion ($p < 0.001$), as shown in Table 2. The significant difference of the serum level of trypsin with or without inflammation effusion indicated that the serum trypsin level was related with inflammation effusion. So, the serum level of trypsin is able to judge whether the inflammation effusion happens. According to the results of Table 2, the serum levels of trypsin in different types of acute pancreatitis were significantly higher than controls ($p < 0.001$). In addition, Table 2 also showed that the serum levels of trypsin in patients with I-II, III, IVA and IVB acute pancreatitis were significantly higher than controls ($p < 0.001$), indicating that the serum level of trypsin is related to the occurrence of acute pancreatitis.

Table 3 showed that the levels of CGRP in serum of males with acute pancreatitis were higher than in females, but there was no statistical difference ($p > 0.05$), which was in keeping with the result of trypsin in Table 2. Table 3 also showed that the serum level of CGRP of patients with inflammation effusion was significantly higher than patients without inflammation effusion ($p < 0.001$), which was in accord with the result of trypsin in Table 2 and can also be used to judge whether the inflammation effusion occurs. Meanwhile, the serum level of CGRP in patients with different types of acute pancreatitis was significantly higher than controls ($p < 0.001$), as shown in Table 3. As shown in Table 3 the serum levels of CGRP in controls were significantly lower than patients with I-II, III, IVA and IVB acute pancreatitis ($p < 0.001$). Thus, according to the results of Table 2 and Table 3, the serum levels of trypsin and CGRP are all related to the occurrence of acute pancreatitis.

CONCLUSION

In conclusion, the serum levels of trypsin and CGRP in patients with acute pancreatitis were always higher than the controls, so we concluded that trypsin and CGRP in serum can act as a new detection index of acute pancreatitis happening. Meanwhile, the serum levels of trypsin and CGRP in patients with acute pancreatitis is able to judge whether the inflammation effusion happens.

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Declaration of Interest:

All authors declared that there were no potential conflicts of interest.

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