BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2021, VOL. 78, NO. 4, 229–232
https://doi.org/10.1080/09674845.2021.1904691

BIOMEDICAL SCIENCE IN BRIEF

Increased plasma clusterin and miR-21 in acute pancreatitis

H Li[a], X Yang[b], B Cao[c] and J Guan[a]

[a]Department of Emergency Medicine, The Affiliated Hospital of Qingdao University, West Coast Hospital, Huangdao, Shandong, China; [b]Department of Operating Room, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China; [c]Department of Gastroenterology, The Affiliated Hospital of Qingdao University, West Coast Hospital, Huangdao, Shandong, China

ARTICLE HISTORY Received 8 January 2021; Accepted 8 March 2021
KEYWORDS Acute pancreatitis; diagnosis; serum markers; liver functions; clusterin; miR-21

Acute pancreatitis is an inflammatory condition associated with a high complication rate and an increased risk of death. The diagnosis can be made by history, physical examination, and the results of diagnostic tests [1]. During an attack of acute pancreatitis, the elevation of alanine aminotransferase (ALT) to >150 IU/L is predictive of a biliary cause [2]. A meta-analysis indicated that this threefold elevation in ALT has a positive predictive value of 95% in diagnosing acute gallstone pancreatitis [3]. Despite the advances in investigational modalities and research techniques, the exact pathogenesis of acute pancreatitis is still unclear. Therefore, identifying the severe form early is one of the major challenges in managing severe acute pancreatitis.

MiR-21, a multifunctional miRNA with inflammation-related roles, regulates different types of inflammatory mediators and involves in the development of experimental acute pancreatitis in mice. In addition, miR-21-3p expression level correlates with the severity of the disease [4], and miR-21 deficiency protects against caerulein- or L-arginine-induced acute pancreatitis in mice. miR-21 is significantly upregulated in type 1 autoimmune pancreatitis compared to healthy adult and chronic pancreatitis. The number of miR-21-5p positive inflammatory cells was significantly elevated in acute pancreatitis [5], suggesting that miR-21-5p may be involved in the regulation of effector pathways in the pathophysiology of acute pancreatitis, thus differentiating acute pancreatitis from chronic pancreatitis.

Apolipoprotein J/Clusterin is a predominantly secreted glycoprotein induced in several tissues in response to injury. It is overexpressed in the pancreas at the onset of chronic pancreatitis in vivo and in cultured acinar cells in response to various stimuli in vitro, suggesting that clusterin has a regulatory role in the exocrine pancreas [6]. Clusterin is also overexpressed in pancreatic cancer tissues and cell lines, but not in the normal pancreas. In a murine acute pancreatitis model, clusterin is overexpressed in stressed exocrine pancreas during the acute phase of pancreatitis [7], and increases dramatically with severity [8]. Whether serum clusterin levels could be used for the diagnosis of acute pancreatitis is unclear. We therefore hypothesized that levels of clusterin and miR-21 have value in the diagnosis and management of acute pancreatitis.

We tested our hypothesis in 147 patients with acute pancreatitis; 92 males: mean/range 43.6 (18–86) years; 55 females: 41.6 (22–76) years were admitted to the affiliated hospital of Qingdao University within 72 hours after the onset of disease between January 2015 and October 2019. The patients were diagnosed according to the criteria of the revised Atlanta classification [1]. Before admission, all the patients had had abdominal ultrasound to exclude cholecystolithiasis and/or ductal gallstones and splenic and/or portal vein thrombosis. Blood was collected upon admission. Exclusion criteria were pregnancy, age <18 years, malignancy-related acute pancreatitis, history of chronic pancreatitis, hepatic cirrhosis, receiving early intervention or surgery due to abdominal compartment syndrome or other reasons before admission, or severe systemic diseases. Clinical information is required for severity assessment using the Revised Atlanta Classification criteria with mild, moderate, and severe acute pancreatitis [1]. The Ethics committee of the Affiliated Hospital of Qingdao University approved our retrospective single-centre study and an informed written consent was obtained from all subjects before inclusion. Control subjects n = 44) were randomly selected from a general medical outpatient clinic when he/she needed to have a blood sample drawn for other reasons or was willing to provide the blood sample for research purposes.

Peripheral blood samples were collected into EDTA-vacutainers, were placed upright for 20 to 25 min, and centrifuged at room temperature at 600 g for 30 min, and the supernatant was further centrifuged at 24°C at 1500 g for 10 min. Plasma was aliquoted and stored at

CONTACT J Guan qdfyzp@126.com Department of Emergency Medicine, The Affiliated Hospital of Qingdao University, West Coast Hospital, Huangdao, Shandong 266003, China.

–80°C until analyses. Liver function tests (LFTs) bilirubin, ALP, ALT, and AST, and amylase and lipase were measured by standard techniques (Pointe Scientific Inc., Canton, MI, USA). Tests were performed on a ChemWell-T chemistry analyser (Awareness Technology, Palm City, FL, USA).

To detect miR-21 expression, 600 µL of the sample from each participant within 72 hours after the onset of disease was subjected to RNA isolation using a mirVana PARIS RNA isolation kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. The RNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and a 15% denatured polyacrylamide gel. The RNA samples were subjected to a reverse transcription reaction using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's instructions. Subsequently, qPCR was carried out on the serum samples in triplicate using TaqMan 2× Universal PCR Master Mix with no AmpErase UNG (Applied Biosystems, CA, USA) on an ABI 7500 Real-Time PCR system (Applied Biosystems). qPCR amplification conditions were set to an initial cycle of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The cycle threshold (Ct) values were calculated using SDS 2.0.1 software (Applied Biosystems). The control miRNA was U6 small nuclear RNA (snRNA). Primers synthesized by Shanghai Yingjun Biotechnology Company and the oligonucleotides were as follows: miR-21:5′-TAGCTTATCAGACTGAT-3′, reference gene U6, the upstream primer, and 5′-CGCTTCGGCAGCACATATAC-3′. Each sample was, respectively, detected three times. The average expression levels of serum miR-21 were calculated using the 2-ΔCt method relative to the average of U6 snRNA. To determine the fold change of miR-21 relative to miR-21 expressed in normal controls, the expression 2-ΔΔCt was used. The mean Ct value of miR-21 was calculated. Clusterin level was determined by commercial ELISA (BioVendor, Brno, Czech Republic) with a detection limit of 5 ng/ml and a range of 5–160 ng/ml.

Data are given as median and Inter-Quartile Range (IQR). For normally distributed data, the Student t test was used to assess the significance of differences; otherwise, the Mann–Whitney U-test was used. Fisher's exact test or linear trend equation was used to assess the significance of associations for non-continuous variables. Chi-square independence tests were applied for qualitative variables. Receiver operating characteristic (ROC) curves were generated and the area under the ROC curve (AUC) with 95% confidence intervals (CI) were computed to assess the discriminating performance of the candidate markers. All significance tests were two-sided, and the results were considered to be statistically significant at P value <0.05. Data were analysed using SPSS for Windows (Version 12.0, SPSS Inc., Chicago, IL).

There were no significant differences in age (P = 0.364) or sex (P = 0.713) between cases and controls. The time duration between pain onset and hospital admission was 0–6 h in 27 patients, 6–12 h in 41 patients, 12–24 h in 31 patients, 24–48 h in 25 patients and 48–72 h in 13 patients. Sixty-three patients had biliary-originated pancreatitis, 25 overeating-induced pancreatitis, 15 alcohol-induced pancreatitis, 7 hypertriglyceridaemia-related pancreatitis, and 27 idiopathic pancreatitis. There were no acute pancreatitis patients due to drug, metabolic causes, or anatomical abnormalities. On ultrasound estimation, 45 patients had pancreatic oedema, 15 patients had peripancreatic fluid, 18 patients had pancreatic oedema and peripancreatic fluid, and 5 patients had pancreatic or peripancreatic necrosis, and 54 patients had normal findings.

Of the 147 patients, 89 had mild, 42 moderate, and 16 severe disease. The overall in-hospital mortality was 5.8%. Five of 16 patients (31%) with severe acute pancreatitis died of refractory multi-organ failure, of whom 3 died within 48 hours, and 2 died within 12 to 21 days. All patients with mild and moderately severe disease survived. As expected, overall, patients had higher amylase (2.4-fold), lipase (3.5-fold) and LFTs (bilirubin 4-fold) than controls (Table 1), but also increased miR-21 (2.4-fold) and clusterin (1.3-fold). Table 1 also shows

Table 1. Serum markers in acute pancreatitis and controls and differential time duration in acute pancreatitis.

			Acute pancreatitis				
			Time (hours)				
Marker	Controls (n = 44)	Acute panceatitis (n = 147)	0–6 (n = 27)	6–12 (n = 41)	12–24 (n = 31)	24–48 (n = 25)	48–72 (n = 13)
Amylase (UI/L)	97 (56–213)	237 (92–993) [c]	140 (60–245)	152(80–416)	243(97–983)[d]	378 (119–1189)[f]	168 (73–524)
Lipase (U/L)	126 (64–480)	446 (98–1160) [c]	197 (67 –560)	246 (70 – 637)	390 (78 – 772)	515 (91 – 927) [d]	680 (110 – 1202) [f]
AST (U/L)	19 (10–86)	54 (25–257) [a]	49 (16–194)	51 (19–253)	53 (27–277)	65 (29–265)	52(24–256)
ALT (U/L)	15 (10–40)	42(18–110) [a]	39(18–103)	41(19–108)	40(19–112)	46(23–117)	42(18–128)
ALP (U/L)	48 (32–126)	120 (74–206) [b]	114 (67–178)	118 (70–196)	123 (73–204)	121 (74–214)	120 (76–213)
Bilirubin (µmol/L)	7 (6–23)	28 (13–49) [a]	16 (6 – 31)	20 (9 – 40)	29 (14 – 36)	35 (19 –61)	39 (18–50)
miR-21 (fold)	1.0	2.4 (2–11)[b]	2.3 (1.3–9)[a]	3.4 (2–17)[e]	8.0 (3.0–26.0) [f]	2.2 (2–12) [d]	1.3 (0.96–6.5)
Clusterin (µg/ml)	56 (10 – 197)	74 (13 – 314) [a]	58 (11 – 205)	60 (12 – 249)	64 (17 – 298)	83 (15 – 417) [d]	123 (17 – 647)[f]

Data: median with IQR; n = number of subjects. P values Vs control, [a]$P<0.05$,[b]$P<0.01$,[c]$P<0.001$; vs 0–6 h, [d]$P<0.05$,[e]$P<0.01$,[f]$P<0.001$. Other groups vs 0–6 h, $p > 0.05$

changes in these molecules up to 72 hours after admission. Amylase, ALT and AST peaked at 24–48 hours, lipase, clusterin and bilirubin at 48–72 hours, ALP and miR-21 at 12–24 hours. The greatest increase compared to controls was miR-21, at 8-fold higher at 12–24 hours.

Table 2 shows data of the markers according to disease severity. Levels of lipase, ALP, bilirubin miR-21 and clusterin all reflected this spectrum, but only levels of clusterin were significantly trend-specific. However, several markers were altered in mild disease versus severe disease. The ROC curves showed an AUC of 0.91 (0.88–0.99; $p < 0.001$) for clusterin at sensitivity of 0.91 and specificity of 0.62 in distinguishing severe acute pancreatitis from other forms. Levels in severe disease ($p < 0.001$), but not mild disease ($p = 0.084$), were significantly higher than in healthy controls. There was no significant trend difference in miR-21 across the disease spectrum, with levels in severe disease not significantly different from controls.

The dynamic course of acute pancreatitis and the risk of developing systemic complications both encourage researchers to look for new early biomarkers of disease severity. Important criteria for assessing the utility of predictive biomarkers include the time needed to conduct adequate laboratory tests, but also the measurement techniques used. In the early phase of acute pancreatitis, life-threatening organ failure may develop during the first 48 hours from the onset of symptoms. This narrows the therapeutic window and indicates the need for urgent measures to improve prognosis in predicted severe disease

Pancreatic enzyme measurement, principally amylase and lipase, is the 'gold standard' for the diagnosis of acute pancreatitis [9]. Serum amylase levels usually rise within 6 to 24 h, peak at 48 h, and decrease to normal or near normal levels over the next 3 to 7 days. Lipase remains elevated for a longer period than amylase, rising within 4 to 8 h, peaks at 24 h, and decreases to normal or near normal levels over the next 8 to 14 days. However, both correlate poorly with disease severity [10]. Our data effectively confirm these views, and although amylase and lipase were significantly increased, serum amylase peaked relatively late (24–48 hours), lipase even later (48–-72 hours), both having low sensitivity but high specificity. These markers are also of limited value in determining disease severity. Patients with acute pancreatitis, especially biliary acute pancreatitis, could suffer liver injury, as marked by increased LFTs. Although as a whole, LFTs were higher in acute pancreatitis, bilirubin was highest in severe disease, although the sensitivity and specificity of diagnosis was very low. Therefore, we submit that LFTs are of limited value as biomarkers for acute pancreatitis diagnosis.

We present two new findings regarding the pathogenesis of acute pancreatitis. An increase in miR-21 concentrations in systemic circulation has been shown to be an early event in experimental acute pancreatitis, preceding the most severe injury of the pancreas [11]. Our clinical data show increased miR-21 levels to be significantly increased at all time points up to 48 hours, peaking at 12–24 hours, the greatest relative increase of all markers. However, against expectations, levels were highest in those with mild disease, falling in those moderate and further still in severe disease, where levels were no different from those of the controls. We interpret this as evidence that severe disease is of such a profound pathophysiological insult that miR-21 is not generated. Nevertheless, we suggest that miR-21 is a good marker of mild disease.

Clusterin is a secretory glycoprotein that is highly induced in several tissues in response to injury, being expressed in various tissues such as brain, ovary, testis, heart, and blood vessels, from which it may be secreted to the circulation [8–10]. Most attacks of display a self-limiting course, suggesting that pancreatic acinar cells may be able to protect themselves against cellular injury, thus preventing further progression of the disease. In experimental pancreatitis *in vitro* and *in vivo*, clusterin mRNA was expressed after 4 hours

Table 2. Markers related to disease severity.

Analyte	Mild (n = 89)	Moderate (n = 42)	Severe (n = 16)	P-value
Amylase (UI/L)	227 (86–873)	286 (93–1042)	252 (106–948)	*0.104*
Lipase (U/L)	506 (108 – 837) [a]	550 (114 –980)	663 (86 – 1920) [b]	0.138
AST (U/L)	52 (25–236)	54 (26–283)	53 (28–246)	*0.147*
ALT (U/L)	40 (19–109)	45 (22–113)	42 (18–132)	*0.253*
ALP (U/L)	110 (77–183)	123 (84–228)	134 (86–230)	*0.874*
Bilirubin(µmol/L)	26 (13 – 29) [c]	29 (14–37)	39 (17 –59) [d]	0.74
miR-21 (fold)	4.1 (2.8–23.6) [e]	2.7 (1.8–13.5)	1.2 (1.0–5.7) [f]	0.156
Clusterin (µg/ml)	59 (14 – 193) [g]	71 (19 – 287)	92 (31 – 456) [h]	0.046

PS: a vs b, p = 0.04; c vs d, p = 0.014; e vs f, P< 0.001; g vs h, P< 0.001.

and peaked at 8 and 24 hours, whereas DNA fragmentation peaked at 72 hours, suggesting that clusterin is a defence mechanism of the exocrine pancreas. Like miR-21, we report raised clusterin in acute pancreatitis, with levels peaking late (48–72 hours, as did amylase), and accordingly may be of little value in diagnosing acute pancreatitis, as the relative increases in both amylase and lipase are greater. However, unlike these two markers, and all LFTs, levels of clusterin increased in a significant linear manner with disease severity, suggesting it has a role as a marker in this respect.

We note certain limitations in our data, the principle being that we cannot control the disease severity and time of blood sampling from admission or onset of symptoms. For example, we cannot determine the degree of disease severity in the 31 patients whose sample was taken at 12–24 hours. It could be argued that patients sampled earlier had more adverse signs and symptoms, and so more severe, and conversely that those with minor signs and symptoms, and so mild disease, were sampled later. This question can only be answered in a prospective study where samples are taken at all time points in all patients, requiring a larger sample size.

This work represents an advance in biomedical science because it shows that miR-21 may help improve the early diagnosis of mild acute pancreatitis, and that clusterin may be a marker of the severity of this disease.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the author(s).

References

[1] Banks PA, Bollen TL, Dervenis C, et al. Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62:102–111.
[2] Ammori B, Boreham B, Lewis P, et al. The biochemical detection of biliary etiology of acute pancreatitis on admission: a revisit in the modern era of biliary imaging. Pancreas. 2003;26:e32–e35.
[3] Tenner S, Dubner H, Steinberg W. Predicting gallstone pancreatitis with laboratory parameters: a meta-analysis. Am J Gastroenterol. 1994;89:1863–1866.
[4] Carroll J, Herrick B, Gipson T, et al. Acute pancreatitis: diagnosis, prognosis and treatment. Am Fam Physician. 2007;75:1513–1520.
[5] Ma X, Conklin DJ, Li F, et al. The oncogenic microRNA miR-21 promotes regulated necrosis in mice. Nat Commun. 2015;6:7151.
[6] De Silva HV, Harmony JA, Stuart WD, et al. Apolipoprotein J: structure and tissue distribution. Biochemistry. 2020;29:5380–5389.
[7] Xie MJ, Motoo Y, Su SB, et al. Expression of clusterin in human pancreatic cancer. Pancreas. 2002;25:234–238.
[8] Iyer S, Park MJ, Moons D, et al. Clusterin and Pycr1 alterations associate with strain and model differences in susceptibility to experimental pancreatitis. Biochem Biophys Res Commun. 2017;482:1346–1352.
[9] Toouli J, Brooke-Smith M, Bassi C, et al. Guidelines for the management of acute pancreatitis. J Gastroenterol Hepatol. 2002;17:S15–S39.
[10] Manjunath K, Sanjay-Kumar HR, Pradeep BK. Prognostication of acute pancreatitis using simple SIRS score and serum interleukin-6 concentration for effective intervention of severe acute pancreatitis in primary health care. Int J Curr Res. 2017;9:47569–47573.
[11] Vissers RJ, Abu-Laban RB, McHugh DF. Amylase and lipase in the emergency department evaluation of acute pancreatitis. J Emerg Med. 1999;17:1027–1037.