Monocyte chemoattractant protein-1, transforming growth factor-β1, nerve growth factor, resistin and hyaluronic acid as serum markers: comparison between recurrent acute and chronic pancreatitis

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BACKGROUND: Diagnostic parameters that can predict the presence of chronic pancreatitis (CP) in patients with recurrent pain due to pancreatitis would help to direct appropriate therapy. This study aimed to compare the serum levels of monocyte chemoattractant protein-1 (MCP-1), transforming growth factor-β1 (TGF-β1), nerve growth factor (NGF), resistin and hyaluronic acid (HA) in patients with recurrent acute pancreatitis (RAP) and CP to assess their ability to differentiate the two conditions.

METHODS: Levels of serum markers assessed by enzyme-linked immunosorbent assay (ELISA) were prospectively compared in consecutive patients with RAP, CP and in controls, and stepwise discriminant analysis was performed to identify the markers differentiating RAP from CP.

RESULTS: One hundred and thirteen consecutive patients (RAP=32, CP=81) and 78 healthy controls were prospectively enrolled. The mean (SD) age of the patients was 32.0 (14.0) years; 89 (78.8%) were male. All markers were significantly higher in CP patients than in the controls (P<0.001); MCP-1, NGF and HA were significantly higher in RAP patients than in the controls (P<0.001). Stepwise discriminant analysis showed significant difference (P=0.002) between RAP and CP for resistin with an accuracy of 61.9%, discriminant scores of ≤-0.479 and ≥0.189 indicating RAP and CP, respectively. The other markers had no differential value between RAP and CP.

CONCLUSION: Serum resistin is a promising marker to differentiate between RAP and CP and needs validation in future studies, especially in those with early CP.

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KEY WORDS: biological marker; cytokine; nerve growth factor; transforming growth factor pancreatitis; resistin

Introduction

Chronic pancreatitis (CP) is characterized by inflammation and fibrosis of the pancreas resulting in irreversible damage to the organ. CP usually manifests as abdominal pain and loss of exocrine and endocrine pancreatic function and eventually increasing the risk of pancreatic cancer.[1] Conceptually, patients with acute pancreatitis (AP) recover their pancreatic function completely, as do those with two or more episodes of AP without evidence of permanent damage to the organ, defined as recurrent AP (RAP).[2] A variable proportion of patients with AP or RAP may eventually progress to CP, thereby manifesting progressive pancreatic damage and loss of function.[3] The full complement of factors that determine the progression from RAP to CP are poorly understood, but a persistent insult to the organ as happens with continuing alcohol consumption is clearly one. However, not all patients with alcohol abuse eventually develop CP.[4] Patients with CP often present

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with recurrent episodes of pain due to acute inflammatory episodes similar to RAP, especially early in the course of the disease. By definition, such patients already have permanent damage to the organ, but easily identifiable features of CP such as ductal dilatation, calcification, diabetes mellitus or steatorrhea may not be evident in many of them.[5] On the other hand, a reversible reduction in exocrine function or transient hyperglycemia in a patient with AP is not necessarily to imply the presence of underlying CP.[6, 7]

Imaging techniques for evaluating the pancreas, such as endoscopic retrograde cholangiopancreatography (ERCP), ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) usually detect late stages of CP. Endoscopic ultrasonography (EUS) is reported to have a high sensitivity, but may be lack of specificity or tend to over-diagnosis of CP.[8, 9] The secretin-pancreozymin test is considered the gold standard, but is cumbersome, poorly standardized, hardly used outside of research protocols and the exact role of newer simplified versions is unknown.[10] The availability of diagnostic parameters that would predict CP in patients who present with recurrent pain from pancreatitis would be of immense help to direct the appropriate interventions. For example, the option of pancreatic surgery available for patients with severe pain of CP is not appropriate for those with idiopathic RAP. Appropriate timely intervention in RAP might also prevent progression to CP.

Irrespective of the etiology, injury to the pancreas results in hyperproliferation and differentiation of fibroblasts into myofibroblasts with excessive synthesis and secretion of the extracellular matrix (ECM). ECM constitutes of proteins (proteoglycan and hyaluronan), sugars, collagens (fibrillar and non-fibrillar), and elastic fibers, and is important for the healing of wounds, homeostasis and development of cells.[11] The simultaneous remodeling of the ECM, a consequence of the quiescent pancreatic stellate cells (PSCs) getting "activated", lays down fibrosis.[12] Activated PSCs secrete the enzyme matrix metalloproteinases (MMPs), which degrades ECM and tissue inhibitor of matrix metalloproteinase (TIMP), inhibiting MMPs.[12] An imbalance between the degradation and formation of ECM contributes to the abnormal ECM synthesis.[12] The necro-inflammation occurring in pancreatitis releases proinflammatory cytokines, transforming growth factor-β1 (TGF-β1), platelet derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), interleukin-1 (IL-1), and IL-6 which activate PSCs and promote pancreatic fibrosis.[13] The synthesis of glycosaminoglycan hyaluronic acid (HA) and other ECM proteins is stimulated by the presence of TGF-β1.[12, 14] Nerve growth factor (NGF) and its neurotrophin receptor p75 are expressed on the PSCs during CP.[12, 15] The levels of TGF-β1, MCP-1 and resistin are increased during inflammation in CP.[16, 17] HA is a useful predictor of liver fibrosis and is also increased in CP.[5, 18] Evaluation of such markers in the serum of patients would offer an attractive option to differentiate RAP from CP. This study aimed to compare the serum levels of MCP-1, TGF-β1, NGF, resistin and HA in patients with RAP and CP and to assess their predictive ability in differentiating the two conditions.

Methods

Patients and controls

All consecutive patients aged 18 years and above with RAP and CP presenting to the Department of Gastroenterology and Hepatology, Kasturba Hospital, Manipal between March 2011 and February 2013 for initial evaluation were qualified for the study. The study was approved by the Ethics Committee of Manipal University. Written informed consent was obtained from each participant. An episode of pancreatitis was defined by the presence of any two of typical upper abdominal pain, raised serum amylase and/or lipase and imaging evidence of pancreatitis. CP was diagnosed by the presence of pancreatic calcifications and/or ductal changes, visualized by US, CT, EUS, ERCP or MRCP; RAP was defined as more than one episode of AP in the absence of changes of CP. Patients with pancreatic cancer were excluded. Patients consuming ≥50 g of alcohol/day were considered to have alcoholic pancreatitis.[19] Patients with gallstones, hypercalcemia and hypertriglyceridemia were excluded from this study. Healthy controls matched for age were included to compare the serum markers with those in the patient group.

Sample collection

Three mL of whole blood was drawn by venipuncture into sterile vacutainer tubes (Becton Dickinson, New Jersey, USA) with no additive or clot activator. The tubes were allowed to stand undisturbed at room temperature for 15-30 minutes, centrifuged at 2000×g for 10 minutes the supernatant (serum) pipetted, apportioned into 0.5 mL aliquots and stored at -80 ℃ until enzyme-linked immunosorbent assay (ELISA) measurement. Stool samples were collected and immediately stored in a -80 ℃ freezer until analysis.

Exocrine and endocrine function tests

Fecal elastase-1 was assessed using the monoclonal