

## Study on chronic pancreatitis and pancreatic cancer using MRS and pancreatic juice samples

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**Author contributions:** Wang J and Ma C contributed equally to this work and performed the majority of experiments and data analysis; Liao Z provided the vital reagents and analytical tools; Tian B collected of pancreatic juice samples; Lu JP designed the study and wrote the manuscript.

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The parameters were as follows: spectral width, 15 KHz; time domain, 64 K; number of scans, 512; and acquisition time, 2.128 s.

**RESULTS:** The main component of pancreatic juice included leucine, iso-leucine, valine, lactate, alanine, acetate, aspartate, lysine, glycine, threonine, tyrosine, histidine, tryptophan, and phenylalanine. On performing 1D  $^1\text{H}$  and 2D total correlation spectroscopy, we found a triplet peak at the chemical shift of 1.19 ppm, which only appeared in the spectra of pancreatic juice obtained from patients with alcoholic chronic pancreatitis. This triplet peak was considered the resonance of the methyl of ethoxy group, which may be associated with the metabolism of alcohol in the pancreas.

**CONCLUSION:** The triplet peak, at the chemical shift of 1.19 ppm is likely to be the characteristic metabolite of alcoholic chronic pancreatitis.

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### Abstract

**AIM:** To investigate the markers of pancreatic diseases and provide basic data and experimental methods for the diagnosis of pancreatic diseases.

**METHODS:** There were 15 patients in the present study, among whom 10 had pancreatic cancer and 5, chronic pancreatitis. In all patients, pancreatic cancer or chronic pancreatitis was located on the head of the pancreas. Pathology data of all patients was confirmed by biopsy and surgery. Among the 10 patients with pancreatic cancer, 3 people had a medical history of long-term alcohol consumption. Of 5 patients with chronic pancreatitis, 4 men suffered from alcoholic chronic pancreatitis. Pancreatic juice samples were obtained from patients by endoscopic retrograde cholangiopancreatography. Magnetic resonance spectroscopy was performed on an 11.7-T scanner (Bruker DRX-500) using Call-Purcell-Meiboom-Gill pulse sequences.

**Key words:** Pancreatic juice; Pancreatic cancer; Chronic pancreatitis; Magnetic resonance spectroscopy; Magnetic resonance imaging

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### INTRODUCTION

Pancreatic cancer accounts for about 2% of all cancer cases, but it has the worst prognosis of all cancers with a 5-year

survival rate of less than 3%<sup>[1,2]</sup>. Because of the deep-seated location of the pancreas and no apparent symptoms at the initial stages of pancreatic cancer, it is difficult to diagnose this disease in the early stages. Chronic pancreatitis is a kind of localized or diffuse inflammation, and is caused by many factors. One of the medical dilemmas is to distinguish pancreatic cancer from chronic pancreatitis with a mass in the head of the pancreas; both these diseases have similar clinical behavior and imaging features<sup>[3]</sup>. A puncture biopsy is usually preferred over an operation when diagnosing the disease, but this is an injurious procedure and may lead to some complications<sup>[4]</sup>.

Magnetic resonance imaging (MRI) is the most common procedure used in the diagnosis of pancreatic cancer. An MRI helps obtain images of the pancreas and its surrounding structures<sup>[5]</sup>. The deep-seated location of the pancreas and similar clinical manifestations of chronic pancreatitis and pancreatic cancer are the main barriers in differentiating between these two diseases even with advanced MRI techniques. Magnetic resonance spectroscopy (MRS) has high sensitivity and resolution, allows in vitro testing of metabolites, and has been widely used in the field of metabolomics<sup>[6-12]</sup>. MRS will be the most potent tool to help differentiate between pancreatic cancer and chronic pancreatitis, and it will be the most effective tool for the early diagnosis of a pancreatic tumor.

Usually, in the process of cancerization, gene and metabolite abnormalities appear before tissue structure transformation. Detection of abnormalities in metabolites facilitates early diagnoses of tumors. Clinically, the serum marker CA19-9<sup>[13]</sup> and gene tumor markers such as the K-ras gene<sup>[14,15]</sup>, p53 anti-oncogene, and p53 protein<sup>[16-19]</sup> are widely used as markers of pancreatic cancer; however, these markers are not sensitive, show low specificity, and are used as auxiliary tools<sup>[20]</sup>. Beger *et al.*<sup>[10]</sup> had successfully used MRS and mass spectrum (MS) to analyze blood constituents of patients with pancreatic cancer and of healthy volunteers; they were able to make a good distinction between pancreatic cancer and the control group by performing lipid profiling of the blood. Pancreatic juice is the exocrine of the pancreas, and is closely related with pancreatic tissues. We wanted to investigate whether it is possible to obtain some information to help differentiate between chronic pancreatitis and pancreatic cancer by analyzing pancreatic juice samples. In the present study, we used MRS technology to analyze the pancreatic juice of patients with pancreatic cancer or chronic pancreatitis with a mass in the head of the pancreas and tried to explore the markers of these diseases. We provided basic data and experimental methods for the study of pancreatopathy.

## MATERIALS AND METHODS

The initial subject population comprised 35 patients with pancreatic cancer (24 men and 11 women; mean age, 67.2 years; age range, 47-85 years) recruited between January 2006 and June 2009. We selected 10 subjects (7 men and 3 women; mean age, 67.7 years; age range, 57-74 years) with surgically confirmed pancreatic cancer. The mean maxi-

mum lesion diameter was 26.1 mm (range, 11-51 mm), and all lesions were located in the head of the pancreas. We chose 5 patients (4 men and 1 woman; mean age, 58.3 years) with chronic pancreatitis, confirmed by in vivo biopsy, and lesions located at the head of the pancreas. The medical history of every patient was recorded in detail. All study protocols were approved by our Institutional Review Board, and informed consent was obtained from all patients before they were enrolled in this study.

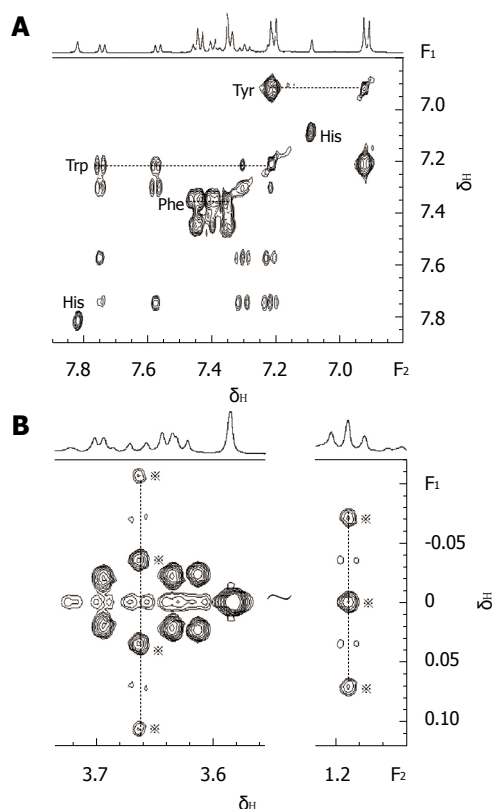
Pancreatic juice samples were obtained from patients by endoscopic retrograde cholangio-pancreatography (ERCP) in frozen tubes and immediately placed in liquid nitrogen before storing the tubes in a -80°C refrigerator for MRS experiments. All patients were diagnosed by biopsy analyses of pathology data. Both the tumor marker CA19-9 and the cancer gene maker p53 were detected in patients with pancreatic cancer, whereas these markers were not detected in patients with chronic pancreatitis.

## MRS experiments

MRS experiments were performed on a Bruker DRX-500 spectrometer (<sup>1</sup>H frequency, 500.13 MHz; Bruker Biospin, Rheinstetten, Germany). Pancreatic juice samples were diluted with phosphate buffer in D<sub>2</sub>O and placed in sample tubes (diameter, 5 mm), which are used in MRS experiments. Spectra were acquired at 300.0 K using Call-Purcell-Meiboom-Gill (CPMG) pulse sequence<sup>[21,22]</sup> along with water presaturation during the relaxation delay of 2 s. The CPMG pulse sequence was applied as a T<sub>2</sub> filter to suppress signals from molecules with short T<sub>2</sub> values (such as macromolecules and lipids), using a total echo time (TE) time of 320 ms. The main parameters for the 1D <sup>1</sup>H-MRS spectra were as follows: spectral width (SW), 15 KHz; time domain (TD), 64 K; number of scans (NS), 512; and acquisition time (AQ), 2.128 s. Spectral assignments were confirmed by 2D <sup>1</sup>H-<sup>1</sup>H TOCSY<sup>[23]</sup> and J-resolved (JRES) along with the values obtained from the literature<sup>[24]</sup>. The main parameters used for TOCSY were as follows: TD (F1-dimensional), 512; TD, (F2-dimensional), 1 K; SW (F1 and F2-dimensional), 5 KHz; and NS, 32. The main parameters used for JRES were as follows: TD (F1-dimensional), 256; TD (F2-dimensional), 8 K; SW (F1-dimensional), 78 Hz; SW (F2-dimensional), 8 KHz; and NS, 32. In both the 2D MRS experiments, the delay time was 2 s. The stability of the pancreatic juice samples was evaluated by repeating a 1D MRS experiment after overall acquisition. No biochemical degradation of any of the pancreatic juice samples was observed.

## RESULTS

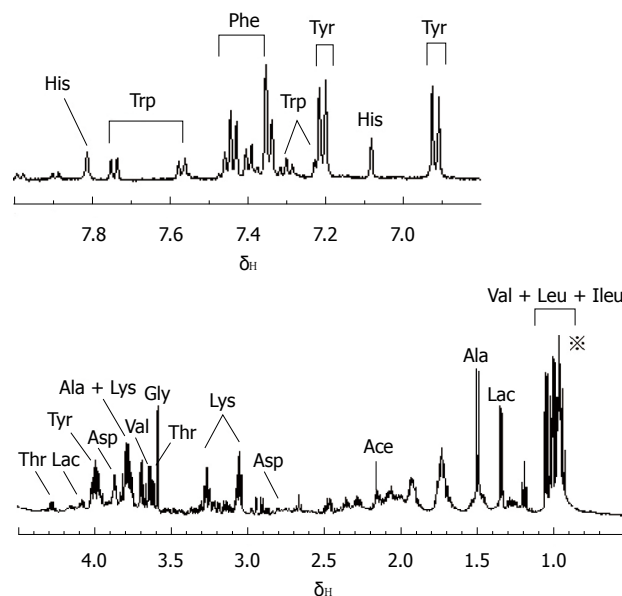
On combining 2D MRS experimental results (Figure 1) obtained in the present study with results from related literature<sup>[25-27]</sup> we identified the main resonances in <sup>1</sup>H MRS spectra of pancreatic juice. TOCSY is a useful 2D MRS technology, it can be used to distinguish the frequencies in the total spin system and improve the sensitivity of detecting small J couplings<sup>[28]</sup>. On the basis of TOCSY data, the resonances in 1D <sup>1</sup>H MRS spectra (Figure 2) of some



**Figure 1** Assignments of partial resonances in  $^1\text{H}$  spectra of pancreatic juice. A: 2D Total correlation spectroscopy (TOCSY) of human pancreatic juice; B: JRES spectra of human pancreatic juice. Tyr: Tyrosine; His: Histidine; Trp: Tryptophan; Phe: Phenylalanine.

amino acids were identified. The components, including leucine (Leu), iso-leucine (Ileu), valine (Val), lactate (Lac), alanine (Ala), acetate (Ace), aspartate (Asp), lysine (Lys), glycine (Gly), threonine (Thr), tyrosine (Tyr), histidine (His), tryptophan (Trp) and phenylalanine (Phe), and the locations were also identified.

From the analysis of 1D  $^1\text{H}$  MRS spectra of all pancreatic juice samples, it was easy to find a triplet peak at the chemical shift of 1.19 ppm (Figure 3), which only appeared in some spectra. Four of the pancreatic juice samples of patients with chronic pancreatitis showed a triplet peak at the chemical shift of 1.19 ppm on  $^1\text{H}$  MRS, whereas the spectra of the pancreatic juice of patients with pancreatic cancer did not show a peak at the chemical shift of 1.19 ppm. When subjected to 2D TOCSY, the spectra of pancreatic juice samples of patients with chronic pancreatitis only showed one correlation peak at the chemical shift of 1.19 ppm and 3.36 ppm (Figure 4). By chemical shift and J coupling constant, we found that the peak at the chemical shift of 1.19 ppm was the  $^1\text{H}$  peak of the methyl of ethoxy group ( $\text{CH}_3\text{CH}_2\text{O}-$ ). The 1D  $^1\text{H}$  spectra of the 4 pancreatic juice samples of patients with chronic pancreatitis showed a triplet peak at the chemical shift of 1.19 ppm; further, these patients had a history of drinking, which was found from the analysis of pathology data. The 1D  $^1\text{H}$  spectra of pancreatic juice obtained from the female patient with chronic pancreatitis did not show a triplet peak at the chemical shift of 1.19 ppm.



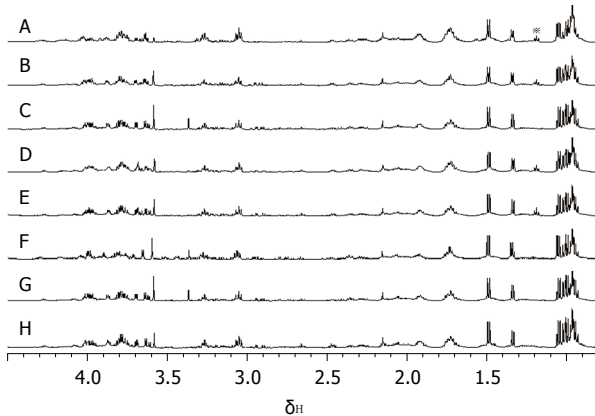
**Figure 2** The assignment of proton magnetic resonance spectroscopy spectra of pancreatic juice with chronic pancreatitis. Ace: Acetate; Ala: Alanine; Asp: Aspartate; Gly: Glycine; His: Histidine; Ileu: Iso-leucine; Lac: Lactate; Leu: Leucine; Lys: Lysine; Phe: Phenylalanine; Thr: Threonine; Tyr: Tyrosine; Trp: Tryptophan; Val: Valine.

Among the 10 patients with pancreatic cancer, diagnosed by postoperative analyses of pathology data, 3 people had a medical history of long-term alcohol consumption. Of 5 patients with chronic pancreatitis, 4 men had a medical history of long-term alcohol consumption, and they were typical patients with alcoholic chronic pancreatitis. The female patient suffered from auto-immune chronic pancreatitis by analyses of the pathology data.

## DISCUSSION

In recent years, there has been much debate on the relation between chronic pancreatitis and pancreatic cancer. While some scholars<sup>[29]</sup> believe that both diseases have a close connection, others<sup>[30]</sup> disagree. The results of our study show that there is no apparent difference between the components of pancreatic juice obtained from patients with chronic pancreatitis and pancreatic cancer, except that 1D  $^1\text{H}$  spectra of pancreatic juice obtained from the former group of patients who suffered from alcoholic chronic pancreatitis shows a triplet peak of the methyl of ethoxy group ( $\text{CH}_3\text{CH}_2\text{O}-$ ) at the chemical shift of 1.19 ppm. This finding may be used to differentiate pancreatic cancer from alcoholic chronic pancreatitis with a mass in the head of the pancreas.

In the present study of the 10 patients with pancreatic cancer, 3 had a medical history of long-term alcohol consumption, but the 1D  $^1\text{H}$  spectra of their pancreatic juice did not show the triplet peak of the ethoxy group ( $\text{CH}_3\text{CH}_2\text{O}-$ ); further, the pathology data of these patients did not show symptoms related to chronic pancreatitis. Hence, we can conclude that alcohol is not the main factor that causes pancreatic cancer. It is controversial whether



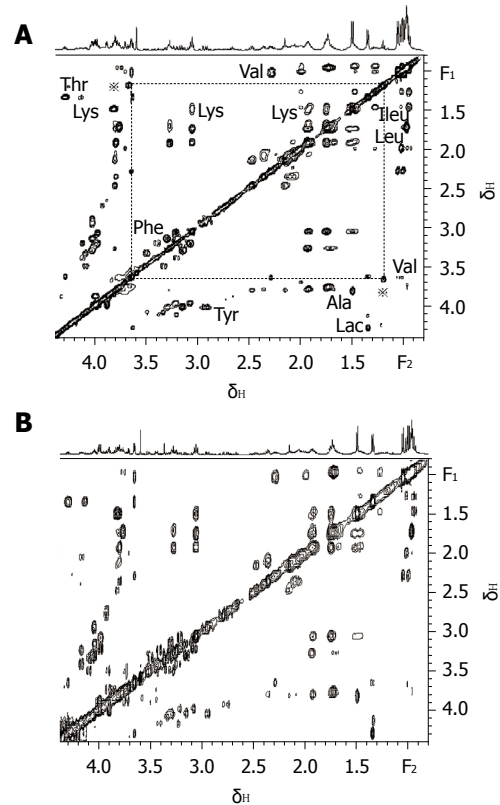
**Figure 3**  $^1\text{H}$  magnetic resonance spectroscopy spectra of human pancreatic juice with chronic pancreatitis and pancreatic cancer. A-E: 1D  $^1\text{H}$  MRS spectra of pancreatic juice of patients with chronic pancreatitis; F-H: 1D  $^1\text{H}$  magnetic resonance spectroscopy spectra of pancreatic juice of patients with pancreatic cancer, other 7 similar spectra of pancreatic juice of patients with pancreatic cancer are not shown.

long-term alcohol consumption can cause pancreatic cancer. Riediger *et al.*<sup>[31]</sup> found that the association between alcohol and pancreatic cancer was not apparent during epidemiological investigation, which is in accord with our results.

We applied MRS to study pancreatic juice obtained from patients with chronic pancreatitis and pancreatic cancer, and separated the various components of different amino acids in human pancreatic juice by 1D and 2D  $^1\text{H}$  spectra. Recently, many analyses on the components of pancreatic juice have focused on the aspect of proteomics. Our study in the field of metabolomics is auxiliary to proteomics, and goes a step further in the study of pancreatopathy.

It is difficult to differentiate between pancreatic cancer and chronic pancreatitis with a mass in the head of the pancreas solely by MRI, because both these diseases have similar clinical behaviors and imaging features. There is no noninvasive method that can be successfully used to diagnose these diseases. Biopsy is frequently used to diagnose pancreatic cancer or chronic pancreatitis with a mass in the head of the pancreas but has some disadvantages, e.g. false negative results, many complications (bleeding, seepage of bile and pancreatic juice), and risk of tumor metastasis. ERCP causes some injury, but it is better than biopsy, because it causes fewer complications. ERCP, when combined with MRS technology, helps obtain more information on metabolites, which cannot be obtained from biopsy or MRI, and is likely to be used to distinguish pancreatic cancer from alcoholic chronic pancreatitis with a mass in the head of pancreas on the basis of  $^1\text{H}$  MRS spectra of pancreatic juice.

There are some limitations to this study. Lack of control groups and the content of metabolites in the pancreatic juice being small meant we could not perform a quantitative analysis, and only obtained some qualitative results. The excreta of patients with pancreatic cancer or chronic pancreatitis may not only have different components but also differ in quantity.



**Figure 4** 2D total correlation spectroscopy spectra of pancreatic juice with chronic pancreatitis and pancreatic cancer. A: Total correlation spectroscopy (TOCSY) spectra of pancreatic juice from patients with alcoholic chronic pancreatitis; B: TOCSY spectra of pancreatic juice from patients with pancreatic cancer. Ala: Alanine; Ile: Iso-leucine; Lac: Lactate; Leu: Leucine; Lys: Lysine; Phe: Phenylalanine; Thr: Threonine; Tyr: Tyrosine; Val: Valine.

In conclusions, MRS is a powerful tool that can be applied to the study of pancreatic juice obtained from patients by ERCP, and does not cause injury. The triplet peak, which is at the chemical shift of 1.19 ppm in 1D  $^1\text{H}$ -MRS data of pancreatic juice obtained from the patients with alcoholic chronic pancreatitis, was identified as resonance of the methyl of ethoxy group ( $\text{CH}_3\text{CH}_2\text{O}-$ ). The ethoxy group may be associated with alcohol metabolism in the pancreas, and is likely to be used to distinguish pancreatic cancer from alcoholic chronic pancreatitis with a mass in the head of the pancreas. In view of small numbers, further confirmation of the results in a larger number of patients is required.

## COMMENTS

### Background

Pancreatic cancer is a malignant neoplasm of the pancreas, and it has a high death rate. One of the medical dilemmas is to distinguish pancreatic cancer from chronic pancreatitis with a mass in the head of the pancreas; both diseases have similar clinical behavior and imaging features. Exploring the markers to distinguish the diseases is very important to the therapies of patients in clinic.

### Research frontiers

CA19-9, *K-ras* gene, p53 anti-oncogene, and p53 protein are widely used as markers of pancreatic cancer, but they are not sensitive and show low specificity. Searching characteristic markers of the diseases is still the goal of tireless pursuit. Magnetic resonance spectroscopy (MRS) has high sensitivity and



resolution, allows in vitro testing of metabolites, and has been widely used in the field of metabolomics. MRS will be the most potent tool to help differentiate between pancreatic cancer and chronic pancreatitis.

### Innovations and breakthroughs

It is difficult to differentiate between pancreatic cancer and chronic pancreatitis with a mass in the head of the pancreas solely by Magnetic Resonance Imaging (MRI). Biopsy is frequently used to diagnose the diseases but has many complications and risk of tumor metastasis. Endoscopic retrograde cholangio-pancreatography (ERCP) combined with MRS helps obtain more information on metabolites, which cannot be obtained from biopsy or MRI. We separated the various components of different amino acids in human pancreatic juice. The triplet peak which is at the chemical shift of 1.19 ppm in 1D  $^1\text{H}$  MRS spectra was identified as resonance of the methyl of ethoxy group ( $\text{CH}_3\text{CH}_2\text{O}-$ ), and it may be the characteristic metabolites of the patients with alcoholic chronic pancreatitis.

### Applications

This study provides basic data and experimental methods for the diagnosis of pancreatic diseases. The ethoxy group is likely to be used to distinguish pancreatic cancer from alcoholic chronic pancreatitis with a mass in the head of the pancreas.

### Terminology

Chemical shift is a basic concept in MRS, the peak appearing in the  $^1\text{H}$  MRS spectra with different chemical shift means the nuclear of proton spins with different frequency. The same rotation frequency of nuclear of protons only shows one peak in the  $^1\text{H}$  MRS spectra. The triplet peak at the chemical shift of 1.19 ppm is caused by the interaction of nearby nuclear of protons. In fact, it is split by one peak.

### Peer review

In my opinion, the article is acceptable for publication.

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