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Original Research Article

CHEMICAL ANALYSIS OF PANCREATIC CALCULI USING FT-IR SPECTROSCOPY IN PATIENTS UNDERGOING FREY'S PROCEDURE: A RETROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: Pancreatic calculi are a characteristic feature of chronic calcific pancreatitis and contribute to ductal obstruction, recurrent inflammation, and pain. Data regarding their chemical composition in Indian patients remain limited. The objective is to characterize the chemical composition of pancreatic calculi using Fourier-transform infrared (FT-IR) spectroscopy in patients undergoing Frey's procedure for chronic calcific pancreatitis.

Materials and Methods: This retrospective observational study included 48 patients with chronic calcific pancreatitis who underwent Frey's procedure between January 2018 and December 2022 at a tertiary care centre in South India. One representative pancreatic stone specimen from each patient was analysed using FT-IR spectroscopy. Demographic characteristics, disease aetiology, stone composition, operative details, and postoperative outcomes were reviewed from institutional records. Descriptive statistics were used for data analysis.

Results: The mean age was 32.0 ± 9.4 years, and 87.5% of patients were male. Tropical pancreatitis was the most common aetiology (58.3%). FT-IR spectroscopy identified five compositional categories. Calcite and Calcite + Gypsum + Protein were the predominant stone types, each 39.6% of specimens, followed by Dolomite (16.7%). Calcium and carbonate were detected in 97.9% of specimens, whereas proteinaceous components were identified in 41.7%. A bimodal distribution of simple mineral stones (56.3%) and complex composite stones (39.6%) was observed. Excellent or good spectral quality was achieved in 98.0% of samples. Mean pain scores improved from 6.2 ± 1.8 preoperatively to 2.1 ± 1.2 at 12 months. Biochemical leaks occurred in 4.2% of patients. No mortality or stone recurrence was observed during follow-up.

Conclusion: Pancreatic calculi in chronic calcific pancreatitis were predominantly composed of calcium carbonate-containing compounds. FT-IR spectroscopy enabled reliable stone characterisation and identified distinct compositional patterns. Frey's procedure was associated with favourable clinical outcomes and effective pain relief.

Keywords: Chronic Pancreatitis; Pancreatic Calculi; Spectroscopy, Fourier Transform Infrared; Calcium Carbonate; Pancreaticojejunostomy; Pancreas.

INTRODUCTION

Chronic pancreatitis is a progressive fibro-inflammatory disease of the pancreas, characterised by irreversible structural damage, pancreatic fibrosis, ductal abnormalities, and progressive loss of exocrine and endocrine function. The disease develops as a result of persistent pancreatic injury in genetically

susceptible individuals exposed to environmental and metabolic risk factors. Chronic pancreatitis remains a major cause of chronic abdominal pain, recurrent hospitalisation, malnutrition, diabetes mellitus (DM), and reduced quality of life. Recent international guidelines continue to recognise pancreatic calcification and intraductal stone formation as important characteristics of advanced disease and indicators of long-standing pancreatic injury.[1,2]

Pancreatic calculi are identified in approximately 50–90% of patients with established chronic pancreatitis and contribute significantly to ductal obstruction, increased intraductal pressure, recurrent inflammation, and pain.[3] The prevalence of pancreatic stone disease is particularly high in tropical calcific pancreatitis, a distinct form of chronic pancreatitis commonly reported in South India and other tropical regions. Tropical pancreatitis is characterised by a younger age at onset, extensive pancreatic calcification, large intraductal calculi, and early development of endocrine insufficiency.[4,5] Alcohol-related chronic pancreatitis remains an important aetiological factor worldwide, and tropical pancreatitis continues to represent a significant clinical burden in many parts of India.

The pathogenesis of pancreatic stone formation is complex and incompletely understood. Current evidence suggests that pancreatic lithogenesis results from interaction of protein plug formation, altered pancreatic juice composition, ductal stasis, calcium supersaturation, inflammation, and genetic factors. Reduced secretion of pancreatic stone proteins and precipitation of calcium salts promote nucleation and stone growth within the pancreatic ducts. Pancreatic calculi differ from urinary calculi in composition and mechanism of formation.[6]

Pancreatic calculi have been analysed using X-ray diffraction, scanning electron microscopy, and chemical assays. These methods have limitations in identifying mixed organic and inorganic components and minor constituents of calculi. Fourier-transform infrared (FT-IR) spectroscopy is a useful technique for biomineral characterisation. It allows rapid, non-destructive identification of inorganic minerals and organic matrix components within a single specimen. FT-IR spectroscopy has been used for characterisation of biological materials and pathological calcifications and is useful for analysis of complex calculi.[7]

Data on pancreatic stone composition remain limited, particularly studies using FT-IR spectroscopy on surgically retrieved calculi. Frey's procedure, which combines local pancreatic head resection with longitudinal pancreaticojejunostomy, is an established treatment for painful chronic calcific pancreatitis with ductal dilatation. It provides ductal decompression and long-term pain relief in selected patients.[8] Surgical retrieval of pancreatic calculi during Frey's procedure provides an opportunity for detailed compositional analysis in a well-defined patient population.

This study aimed to analyse the chemical composition of pancreatic calculi using FT-IR spectroscopy in patients undergoing Frey's procedure for chronic calcific pancreatitis at a tertiary care centre in South India.

Objectives

To evaluate the distribution of individual chemical components and describe stone composition, disease aetiology, and surgical outcomes.

MATERIALS AND METHODS

This retrospective observational study was conducted at the Department of Surgical Gastroenterology, Kanyakumari Government Medical College Hospital, Nagercoil, Tamil Nadu, India. The study included patients diagnosed with chronic calcific pancreatitis who underwent Frey's procedure between January 2018 and December 2022. Clinical records, operative details, and stone analysis reports were reviewed from an institutional database.

Inclusion criteria

Patients aged ≥18 years with chronic pancreatitis and radiological evidence of pancreatic calcification who underwent Frey's procedure and had pancreatic calculi retrieved during surgery were included in the study.

Exclusion criteria

Patients with acute pancreatitis, pancreatic malignancy, secondary causes of pancreatitis, previous pancreatic surgery, or incomplete clinical records were excluded.

Sample size: A total of 48 patients with chronic calcific pancreatitis who underwent Frey's procedure and had pancreatic calculi available for FT-IR analysis were included in the study. Four non-pancreatic renal stone specimens received during the study period were analysed separately for laboratory quality comparison and excluded from all statistical analyses.

Methods: A detailed review of the hospital records was performed. Demographic data, including age, sex, disease duration, alcohol consumption, smoking history, and aetiology of chronic pancreatitis, were collected. Clinical parameters, including the history of acute pancreatitis, DM, and exocrine pancreatic insufficiency, were recorded.

Preoperative laboratory, radiological, operative, and follow-up data were obtained from medical records. Operative variables included duration of surgery, blood loss, number of stones retrieved, and total stone weight. Postoperative complications were graded using the Clavien-Dindo classification. Pancreatic fistulas were classified according to International Study Group on Pancreatic Fistula (ISGPF) criteria.

Pancreatic calculi retrieved during Frey's procedure were collected and sent to Apollo Diagnostics for chemical composition analysis. The calculi were dried, powdered, and analysed using Fourier-transform infrared (FT-IR) spectroscopy (Bruker IFS 66/S). Analysis was performed over a scanning range

of 400–4000 cm^{-1} with a spectral resolution of 4 cm^{-1} using the potassium bromide pellet method.

Spectral matching was performed using validated reference libraries to identify stone components. Laboratory personnel performing FT-IR analysis were blinded to the clinical and operative details. One representative stone specimen from each patient was analysed.

Patients were followed for 12 months after surgery. Pain relief was assessed using the Visual Analogue Scale (VAS). Recurrence of pancreatic calculi was recorded when available.

Statistical analysis: Statistical analysis was performed using SPSS version 26. Continuous variables were expressed as mean ± standard deviation and categorical variables as frequency and percentage.

Ethical considerations: The study protocol was reviewed and approved by the Institutional Ethics Committee of Kanyakumari Government Medical College Hospital. As this was a retrospective record-based study, patient confidentiality was maintained throughout the study, and all collected data were anonymised prior to analysis.

RESULTS

A total of 48 patients with chronic calcific pancreatitis who underwent Frey's procedure and had pancreatic calculi available for FT-IR analysis were included in the study. The mean age was 32.0 ± 9.4 years (range, 20–56 years). Male patients predominated, 87.5% (n=42), whereas females 12.5% (n=6). Tropical pancreatitis was the most common aetiology in 58.3% (n=28) of patients, followed by alcoholic chronic pancreatitis (41.7%).

The mean disease duration was 3.5 ± 1.8 years. The patients experienced a mean of 4.8 ± 1.3 prior episodes of acute pancreatitis. None of the patients had undergone endoscopic retrograde cholangiopancreatography (ERCP), pancreatic duct stenting, or extracorporeal shock wave lithotripsy (ESWL). Alcohol consumption was reported in 41.7% (n=20) and smoking in 54.2% (n=26) of the patients. DM and exocrine pancreatic insufficiency were present in 41.7% (n=20) and 37.5% (n=18) of patients, respectively. The mean preoperative pain score was 6.2 ± 1.8. A total of 283 pancreatic calculi were retrieved, corresponding to a mean of 5.9 stones per patient and an aggregate stone weight of 233.1 g (mean 4.9 g per patient) [Table 1].

Table 1: Baseline Clinical Characteristics (n=48)

Variable	Category	Value
Age (years)		32.0 ± 9.4
Sex	Male	42 (87.5%)
	Female	6 (12.5%)
Disease Characteristics	Tropical pancreatitis	28 (58.3%)
	Alcoholic pancreatitis	20 (41.7%)
	Disease duration (years)	3.5 ± 1.8
	Prior acute pancreatitis episodes	4.8 ± 1.3
	Preoperative VAS score	6.2 ± 1.8
Risk Factors and Comorbidities	Alcohol use	20 (41.7%)
	Smoking	26 (54.2%)
	Diabetes mellitus	20 (41.7%)
	Exocrine pancreatic insufficiency	18 (37.5%)
Stone Characteristics	Total stones retrieved	283
	Mean stones per patient	5.9
	Total stone weight (g)	233.1

FT-IR spectroscopy identified five stone composition patterns among 48 pancreatic calculi. Calcite and Calcite + Gypsum + Protein were the most common, each present in 19 (39.6%) specimens. Dolomite was identified in 8 (16.7%) specimens. Calcium Bilirubinate and Protein + Carbonate Apatite were found in 1 (2.1%) specimen each [Table 2].

Mean stone weight was highest in the Calcite + Gypsum + Protein group (6.75 g), followed by Calcium Bilirubinate (4.91 g), Dolomite (4.66 g), Calcite (3.23 g), and Protein + Carbonate Apatite (1.22 g). Most calculi were brown in colour, whereas Calcite + Gypsum + Protein stones were cream to brown.

Table 2: FT-IR Composition of Pancreatic Calculi (n=48)

Composition	N (%)
Calcite	19 (39.6%)
Calcite + Gypsum + Protein	19 (39.6%)
Dolomite	8 (16.7%)
Calcium Bilirubinate	1 (2.1%)
Protein + Carbonate Apatite	1 (2.1%)

Analysis of stone components showed calcium and carbonate in 47 (97.9%) specimens. Protein components were present in 20 (41.7%) specimens, while sulphate-containing compounds (gypsum) were identified in 19 (39.6%). Magnesium was detected in 8 (16.7%) calculi. Bilirubin was found in only 1 (2.1%) specimen. Uric acid and oxalate were not detected in any specimen [Table 3].

Table 3: Prevalence of Chemical Components

Component	Positive, n (%)
Calcium	47 (97.9%)
Carbonate	47 (97.9%)
Amine/Proteins	20 (41.7%)
Sulphate (Gypsum)	19 (39.6%)
Magnesium	8 (16.7%)
Bilirubin	1 (2.1%)
Uric Acid	0 (0.0%)
Oxalate	0 (0.0%)

A bimodal distribution of pancreatic calculi was observed. Simple mineral stones (Calcite and Dolomite) accounted for 27 (56.3%) cases. Complex stones composed of Calcite + Gypsum + Protein accounted for 19 (39.6%) cases. Atypical compositions were identified in 2 (4.1%) cases. FT-IR spectral matching showed excellent quality in 38 (79.2%) samples, good quality in 9 (18.8%), and fair quality in 1 (2.1%) sample [Table 4].

Table 4: Spectral Quality Distribution

Spectral Quality	n (%)
Excellent (>0.95)	38 (79.2%)
Good (0.90–0.95)	9 (18.8%)
Fair (<0.90)	1 (2.1%)

Among 28 patients with tropical pancreatitis, calcite was the most common stone type, present in 14 (50.0%) cases, followed by Calcite + Gypsum + Protein in 12 (42.9%) and Dolomite in 2 (7.1%) cases. Among 20 patients with alcoholic chronic pancreatitis, Calcite + Gypsum + Protein was the most common composition, present in 11 (55.0%) cases, followed by Calcite in 7 (35.0%) and Dolomite in 2 (10.0%) cases [Table 5].

Table 5: Distribution of Major Stone Compositions According to Aetiology

Composition	Tropical (n=28)	Alcoholic (n=20)
Calcite	14 (50.0%)	7 (35.0%)
Calcite + Gypsum + Protein	12 (42.9%)	11 (55.0%)
Dolomite	2 (7.1%)	2 (10.0%)

Rare stone compositions (Calcium Bilirubinate and Protein + Carbonate Apatite) were excluded from aetiology-wise comparison because only one case of each was identified.

All patients underwent Frey's procedure successfully. Mean operative duration was 185.8 ± 28.3 min and mean blood loss was 124 ± 67 mL. Mean postoperative hospital stay was 5.2 ± 2.1 days. Postoperative complications were uncommon. Four patients (8.3%) developed Clavien-Dindo Grade I complications and four (8.3%) developed Grade II complications. No Grade III, IV, or V complications were observed. Pancreatic fistula occurred in 2 (4.2%) patients, biochemical leaks (Grade A) according to ISGPS criteria. No Grade B or C fistulas occurred, and there was no postoperative mortality.

At the 12-month follow-up, the mean VAS pain score decreased from 6.2 ± 1.8 preoperatively to 2.1 ± 1.2 post-operative. Excellent pain relief (VAS ≤2) was achieved in 77.1% (37/48) of the patients. Good pain relief (VAS 3–4) occurred in 18.8% (9/48), whereas only 4.2% (2/48) experienced moderate residual pain (VAS 5–6).

Radiological follow-up was available for 46 patients (95.8%). No cases of recurrent pancreatic calculi or new ductal obstruction were documented during the 12-month follow-up [Table 6].

Table 6: Twelve-Month Clinical Outcomes

Outcome	Category	Value
Pain Assessment	Preoperative VAS score	6.2 ± 1.8
	12-month postoperative VAS score	2.1 ± 1.2
	Excellent pain relief (VAS ≤2)	37 (77.08%)
	Good pain relief (VAS 3–4)	9 (18.75%)
	Moderate pain relief (VAS 5–6)	2 (4.17%)
Postoperative Complications	Biochemical leak (formerly Grade A pancreatic fistula)	2 (4.2%)
	Grade B/C pancreatic fistula	0
	Mortality	0
Follow-up Outcomes	Radiological follow-up available	46 (95.8%)
	Stone recurrence	0/46 (0%)
	New ductal obstruction	0/46 (0%)

DISCUSSION

The present study evaluated the chemical composition of pancreatic calculi using FT-IR spectroscopy in patients who underwent Frey's procedure for chronic calcific pancreatitis. The major findings were the predominance of calcium carbonate-based calculi, with Calcite and Calcite + Gypsum + Protein for 79.2% of all analysed stones. Calcium and carbonate were identified in 97.9% of the specimens. Similar findings have been reported by Pramanik et al. and Cros et al., who identified calcium carbonate as the principal constituent of pancreatic calculi.[9,10]

In the present study, a distinct bimodal distribution of simple mineral stones and complex protein-containing composite stones was observed. Comparable heterogeneity in stone composition has also been described by Cros et al,[10] and more recently by Wu et al,[6] who suggested the presence of multiple pathogenic pathways involved in pancreatic stone formation. The higher prevalence of protein-containing stones in alcoholic pancreatitis may suggest an association with disease aetiology. Oxalate and uric acid were absent in all pancreatic calculi analysed, supporting the concept that pancreatic calculi differ from urinary calculi in their biochemical composition. Frey's procedure resulted in significant postoperative pain reduction, low morbidity, minimal pancreatic fistula rates, and absence of stone recurrence during follow-up, findings that are comparable to previously reported outcomes.[11-13] Calcite was the most common stone composition 39.6% of cases. Tandan et al. and Wu et al. described calcium carbonate precipitation as a key event in pancreatic stone formation.[6,14] The predominance of calcite-containing calculi in our study supports the central role of calcium carbonate deposition in pancreatic lithogenesis.

In the present study, 39.6% of pancreatic calculi were composed of Calcite + Gypsum + Protein, whereas proteinaceous components were detected in 41.7% of all specimens. These findings suggest that organic matrix components contribute to stone formation in a substantial proportion of patients. De Caro et al. first showed the role of pancreatic stone protein in inhibiting calcium carbonate crystallization.[15] Tandan et al. reported that pancreatic stone formation begins with the development of protein-rich intraductal plugs, which later undergo calcification due to calcium carbonate deposition.[14] Wu et al. reported that protein precipitation is an early event in pancreatic lithogenesis, with progressive calcification around protein nuclei.[6] The presence of gypsum and protein along with calcite in our study suggests a more complex pattern of stone formation than purely mineral calculi.

Cros et al. also reported compositional heterogeneity in pancreatic calcifications.[10] Wu et al. described multiple mechanisms of pancreatic lithogenesis, including mineral precipitation and protein-mediated calcification.[6] These findings support heterogeneity in pancreatic stone formation.

In the present study, tropical pancreatitis was the predominant aetiology in 58.3% of cases. Patients with alcoholic chronic pancreatitis appeared to have a higher proportion of Calcite + Gypsum + Protein stones than those with tropical pancreatitis (55.0% vs. 42.9%). Wu et al. reported that alcohol alters pancreatic juice composition and promotes protein precipitation within the pancreatic ductal system, facilitating subsequent calcification.[6] Eperjesi et al. also identified alcohol consumption as an important risk factor for pancreatic calcification in chronic pancreatitis.[16] The higher frequency of protein-containing composite stones in the alcoholic group observed in our study may indicate differences in stone composition between aetiological groups.

Oxalate and uric acid were absent in all the calculi analysed in the present study. In contrast, calcium oxalate and uric acid are the most common constituents of urinary calculi. Cros et al. reported that pancreatic calcifications are composed predominantly of calcium carbonate minerals with variable organic matrix components, while oxalate-containing stones are rarely identified in the pancreatic ductal system.[10] The absence of oxalate and uric acid in our study supports that pancreatic stone formation differs fundamentally from urinary stone disease and is primarily driven by calcium carbonate deposition within the pancreatic ducts.

FT-IR spectroscopy proved to be a useful technique for the characterisation of pancreatic calculi in the present study. This method enabled the simultaneous identification of both mineral and organic components. Pramanik et al. and Cros et al. showed the utility of this technique in determining the stone composition. In our study, excellent or good spectral quality was achieved in 98.0% of specimens.[9,10] Frey's procedure was associated with favourable postoperative outcomes in the present study. Mean pain scores improved from 6.2 ± 1.8 preoperatively to 2.1 ± 1.2 at 12 months, with 77.1% of the patients achieving excellent pain relief. Postoperative morbidity was low, with only 4.2% of patients developing Grade A pancreatic fistulas and no Grade B/C fistulas or mortality. No recurrence was observed in patients with available follow-up imaging. Previous studies showed effective long-term pain control and low postoperative morbidity following Frey's procedure.[11,12] Dangi et al. reported complete or near-complete pain relief in approximately 75% of patients, comparable to the 77.1% observed in our study.[13]

Liu et al. reported imaging factors associated with ESWL fragmentation efficacy.[17] Kaushik et al. suggested that stone composition analysis may improve understanding of disease mechanisms and aid future therapeutic research.[18] Further studies are needed to determine the clinical significance of different stone compositions in chronic pancreatitis. Pancreatic calculi are predominantly calcium carbonate-based. FT-IR spectroscopy enabled

reliable stone characterisation, and Frey's procedure resulted in favourable clinical outcomes.

Strengths and Limitations: Strengths of this study include FT-IR-based stone analysis, uniform surgical treatment with Frey's procedure, and systematic follow-up. Limitations include the retrospective design, single-centre setting, small sample size, and lack of biochemical or histopathological correlation.

CONCLUSION

Pancreatic calculi were predominantly composed of calcium carbonate-containing compounds. Calcite and Calcite + Gypsum + Protein were the most common compositions. FT-IR spectroscopy identified distinct stone composition patterns. Frey's procedure was associated with favourable surgical outcomes and significant pain reduction. No stone recurrence was observed during follow-up.

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