A STUDY OF SPINK 1 MUTATION AND OTHER CLINICAL CORRELATES IN IDIOPATHIC RECURENT ACUTE PANCREATITIS AND IDIOPATHIC CHRONIC PANCREATITIS

DISSERTATION SUBMITTED IN FULFILLMENT OF THE REGULATIONS FOR THE AWARD OF

D.M (GASTROENTEROLOGY)

DEPARTMENT OF GASTROENTEROLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, TAMILNADU, INDIA

AUGUST– 2012

CERTIFICATE

This is to certify that **Dr. SHIRAN SHETTY** has prepared this dissertation entitled "**A STUDY OF SPINK 1 MUTATION AND OTHER CLINICAL CORRELATES IN IDIOPATHIC RECURENT ACUTE PANCREATITIS AND IDIOPATHIC CHRONIC PANCREATITIS"** under our overall supervision and guidance in the Institute of PSG Institute of Medical Science and Research, Coimbatore in partial fulfillment of the regulations of Tamil Nadu **Dr. M.G.R. Medical University** for the award of **D M Degree in Medical Gastroenterology.**

Dr. L Venkatakrishnan MD DM DNB AFSA
Professor and Head
Department of Gastroenterology
PSGIMS&R
Coimbatore

Dr. S Ramalingam MD
Principal
PSGIMS&R

DECLARATION

I hereby declare that this dissertation entitled "**A STUDY OF SPINK 1 MUTATION AND OTHER CLINICAL CORRELATES IN IDIOPATHIC RECURENT ACUTE PANCREATITIS AND IDIOPATHIC CHRONIC PANCREATITIS"** was prepared by me under the direct guidance and supervision of **Prof. DR. L. VENKATAKRISHNAN MD DM DNB AFSA (FRANCE),** PSG Hospitals, Coimbatore.

The dissertation is submitted to the Dr. M.G.R. Medical University in partial fulfillment of the University regulations for the award of DM degree in Medical Gastroenterology. This dissertation has not been submitted for the award of any Degree or Diploma.

Date: **Dr. SHIRAN SHETTY**

Place:

ACKNOWLEDGEMENT

I wish to express my sincere thanks and gratitude to my P**rof. Dr. L. Venkatakrishnan MD DM DNB AFSA (FRANCE),** Department of Gastroenterology and **Principal Dr. S. Ramalingam** PSG Institute of Medical Sciences & Research for their guidance and encouragement all along in completing my study. They showed me different ways of approach to study the problem and the need to be persistent to accomplish my goal.

I sincerely thank Prof. Dr J Krishnaveni for her guidance and encouragement.I also thank also Dr A Mohanakrishnan and Dr.M.Pazhanivel for their valuable support, suggestions and guidance.

I am thankful to Miss. Seethalakshmi and Dr.Thiyagarajan and Dr.Sudha Ramalingam for their support and guidance in my genetic analysis.

I am so grateful to the **Medical Director, Dr. J.S. Bhuvaneswaran**, PSG Hospitals for permitting me to carry out this study.

I thank **Mrs. V. Revathi** in helping me in typing and editing in this study and also thank all the patients for the kind cooperation and most of all I express gratitude to my family and my friends who helped me all the way through. I am grateful to the institution for providing the necessary financial aid.

CONTENTS

S.NO	CHAPTER	PAGE NO
1.	Introduction	1 - 2
2.	Aims and Objective	3
3.	Review of Literature	4 – 23
4.	Materials and Methods	24 – 32
5.	Observations and Results	33 – 48
6.	Discussion	49 – 52
7.	Summary and Conclusion	53 – 54
8.	Bibliography	
9.	Proforma	
	Colour Plates	

ABBREVIATIONS

CP	-	Chronic Pancreatitis
RAP	-	Recurrent Acute Pancreatitis
TCP	-	Tropical Calcific Pancreatitis
PRSS1	-	Cationic trypsinogen gene
SPINK 1	-	Serine Protease Inhibitor Kazal type 1
CFTR	-	Cystic Fibrosis Transmembrane Conductance Regulator
EUS	-	Endoscopic ultrasonography
CT	-	Computer Tomography
ERCP	-	Endoscopic Retrograde Cholangiopancreatography
MRCP	-	Magnetic Resonance Cholangiopancreatography
RFLP	-	Restrcition Fragment Length Polymorphism
PCR	-	Polymerase Chain Reaction
PAGE	_	Poly Acrylamide Gel Electrophoresis

ABSTRACT

Recurrent acute pancreatitis and chronic pancreatitis are labeled as idiopathic when no identifiable factors are found. The identifications of genetic mutations associated with pancreatitis have provided opportunities for identifying patients at risk for idiopathic pancreatitis.

Aim:

To study of clinical profile and prevalence of SPINK 1 mutation in idiopathic recurrent acute and chronic pancreatitis.

Design:

Prospective observational study of patients with idiopathic recurrent and chronic pancreatitis in a tertiary care hospital from November 2010 to 31st December 2011.

Results:

Fifty patients were included out which 17 patients were idiopathic recurrent acute pancreatitis and 33 were chronic. Out of 17 patients with RAP mean age was 22.29 ± 9.7 years, duration of illness was 28.23 ± 10.34 months, 82% were male, 94% had BMI > 18.5 kg/ m^2 41.17% had SPINK1 mutation. Out of 33 patients with chronic pancreatitis mean age was 31.75 ± 13.07 year, duration of illness was 31.33 ± 19.89 months, mean fasting sugar was 112.57 mg/dl, 67% were male, 93.94% had pain 87.8% had ductal dilatation on CT, 36.36% were SPINK 1 positive.

Conclusion:

SPINK1 mutation patients have more frequent episodes of pancreatitis and parenchymal calcification on CT. The clinical profile of idiopathic chronic pancreatitis is different from what has been reported in the past.

Key words:

Idiopathic Recurrent Acute Pancreatitis; Chronic Pancreatitis SPINK1 mutation

INTRODUCTION

Recurrent pancreatitis is defined as two or more attacks of pancreatitis and chronic pancreatitis is defined as a continuing inflammatory disease of the pancreas characterized by irreversible morphological changes and typically causing pain and permanent loss of function[1,2]. Many studies have been conducted on acute and chronic pancreatitis, but only few have focused on idiopathic chronic and recurrent acute pancreatitis.

In clinical practice evaluation fails to detect the cause of pancreatitis in 10 – 30% of the patients, and these patients are labeled as idiopathic chronic and recurrent acute (RAP) pancreatitis. Evaluation is important in such patients since more than 50% of patients with RAP experience recurrent episodes that can lead to chronic pancreatitis[3].

Very few studies have been conducted on idiopathic recurrent acute pancreatitis. RAP can be due to biliary disease, alcohol, trauma, hypercalcemia, hyperlipedemia, or anatomical variations[2]. In clinical practice upto 30% do not have identificable factors and are labeled as idiopathic recurrent pancreatitis.

Idiopathic chronic pancreatitis was early thought to be seen only in certain parts of India. During the last few years, many cases of idiopathic chronic pancreatitis have been reported from western world and almost all states in our country. Mutations in cationic trypsinogen gene (PRSS1), SPINK1 gene, cystic fibrosis transmembrane conductance regular gene (CFTR) and Cathepsin B gene have been studied in acute recurrent and chronic pancreatitis[4-7].

Genetic mutations may be the cause of pancreatitis in patients whom etiology is not found. Idiopathic pancreatitis represents a complex disease process resulting from an interaction of genetic mutations and environmental factors. Recent research have shown complex interactions like gene –gene, gene – environment in the pathogenesis of pancreatitis[8]. A systematic study on clinical profile and prevalence of SPINK 1 mutation was studied in patients with idiopathic recurrent acute pancreatitis and idiopathic chronic pancreatitis.

AIMS AND OBJECTIVES

To study the demographic and clinical profile of idiopathic recurrent acute pancreatitis and idiopathic chronic pancreatitis.

To assess the prevalence of genetic mutation (SPINK 1) in idiopathic recurrent acute pancreatitis and chronic pancreatitis.

REVIEW OF LITERATURE

Definition:

Acute Pancreatitis: is defined as a acute inflammatory disease of the pancreas presenting with abdominal pain and usually associated with elevated pancreatic enzymes in blood or urine[9].

Recurrent Acute Pancreatitis: is defined as two or more attacks of pancreatitis associated with at least twice normal serum amylase levels[9].

Chronic Pancreatitis: is defined as a continuing inflammatory disease of the pancreas characterized by irreversible morphologic changes that typically causes pain and or permanent loss of exocrine and or endocrine function. Pain is the predominant feature and is associated with pancreatic calcification, diabetes and steatorrhoea[10]. The newer imaging techniques like endoscopic ultrasonography (EUS), endoscopic retrograde pancreatography (ERCP), Magnetic resonance imaging changes are useful in detecting early changes in ducts and parenchyma. The identifications of genetic mutation associated with chronic pancreatitis like SPINK 1, CFTR, PRSS, have provided opportunities for identifying patients at risk for idiopathic pancreatitis[11-13]. This review of literature will focus on epidemiology, classification of chronic pancreatitis, etiopathogenesis and genetic developments in idiopathic recurrent and chronic pancreatitis.

Incidence

The study from China by Wang et al[14] showed that 10.6% of their acute pancreatitis had recurrent episodes. The data from Europe by Gullo et al showed that out of the 1068 of acute pancreatitis, 288 (27%) had recurrence[15].

The incidence of chronic pancreatitis appears to be around 3 – 10 patients

100000 population. The prevalence is now estimated to be around 13 per 100000[16] people. Idiopathic chronic pancreatitis is prevalent both in Western countries and India[17]. Recent study shows that chronic pancreatitis previously classified as tropical pancreatitis represents idiopathic chronic pancreatitis in India. The true incidence and prevalence of chronic pancreatitis in India are not known[18]. This is because studies related to idiopathic pancreatitis have been difficult to do because of insidious onset, difficulty in diagnosis, and the fact that the disease presents often as acute or recurrent pancreatitis without any definite evidence of chronic pancreatitis[19].

Etiology and risk factors[20-30]

The recurrent acute pancreatitis is called as idiopathic when no definite cause is found. The following are the few causes for recurrent acute pancreatitis.

1. Gall stones
2. Biliary sludge and Microlithiasis
3. Alcohol
4. Sphincter Oddi Dysfunctio
5. Pancreas Divisum
6. Drugs
7. Hypertriglyceridemia
8. Hypercalcemia
9. Infection
10. Biliary Ascariasis
11. Trauma
12. Vascular Diseases
13. Tumours

14. Choledochocele

15. Annular Pancreas

16. Genetic Mutations

Chronic Pancreatitis:

The exact etiology is partially known. Ethanol is considered as the commonest cause accounting for more than 50% of case along with other factors like hereditary, environmental anatomical variation, metabolic, genetics. Many classification systems have been proposed for chronic pancreatitis. These classification include

(i) The Marseille classification (1963) and revised Marseille classification in 1984[31-32].

(ii) The Marseille-Rome classification of 1988[33].

(iii) The Cambridge classification of 1984[34].

(iv) The Japan Pancreas Society classification for CP [35].

(v) TIGAR -O classification[36].

TIGAR – 0 classification systems was proposed by Etemad and Whitcomb in 2001 and is based on primarily on the etiology of chronic pancreatitis and takes into account of newer developments such as genetic mutations.

TIGAR -O Classification System for Chronic Pancreatitis

Toxic Metabolic

- Alcoholic
- Tobacco Smoking
- Hypercalcemia
- Hyperlipemia
- Chronic Renal Failure

Idiopathic

- Early onset
- Late onset
- Tropical

Genetic

- Autosomal dominant: cationic trypsinogen gene mutation
- Autosomal recessive / modifiers genes: CFTR mutations, SPINK1 Mutations

Autoimmune

- Isolated autoimmune CP
- Associated with other autoimmune diseases (Sjogren syndrome- associated CP

Recurrent and severe acute pancreatitis

- Post - necrotic (severe acute pancreatitis)
- Recurrent acute pancreatitis
- Vascular disease / ischemic
- Radiation injury

Obstructive

- Pancreas divisum
- Sphincter of Oddi disorder (controversial)
- Duct obstruction (e.g) tumour
- Periampullary duodenal wall cysts
- Post –traumic

PATHOGENESIS

Trypsin and Pancreatitis:

Trypsin is a major pancreatic serine protease with two protein domains connected by a single side chain. Trypsinogen becomes trypsin with cleavage of a short chain exposed peptide chain called trypsinogen activation peptide by the action of enterokinase or by a second trypsin molecule. The trypsin contains a calcium binding pocket near the side chain connecting the globular domains. Enzymatic cleavage of the side chain by the second trypsin leads to destruction of the first trypsin molecule (autolysis). If the concentration of soluble calcium rises, calcium enters the binding pocket and limits exposure to enzymatic attack by another trypsin and prevents trypsin from autolysis[37,38].

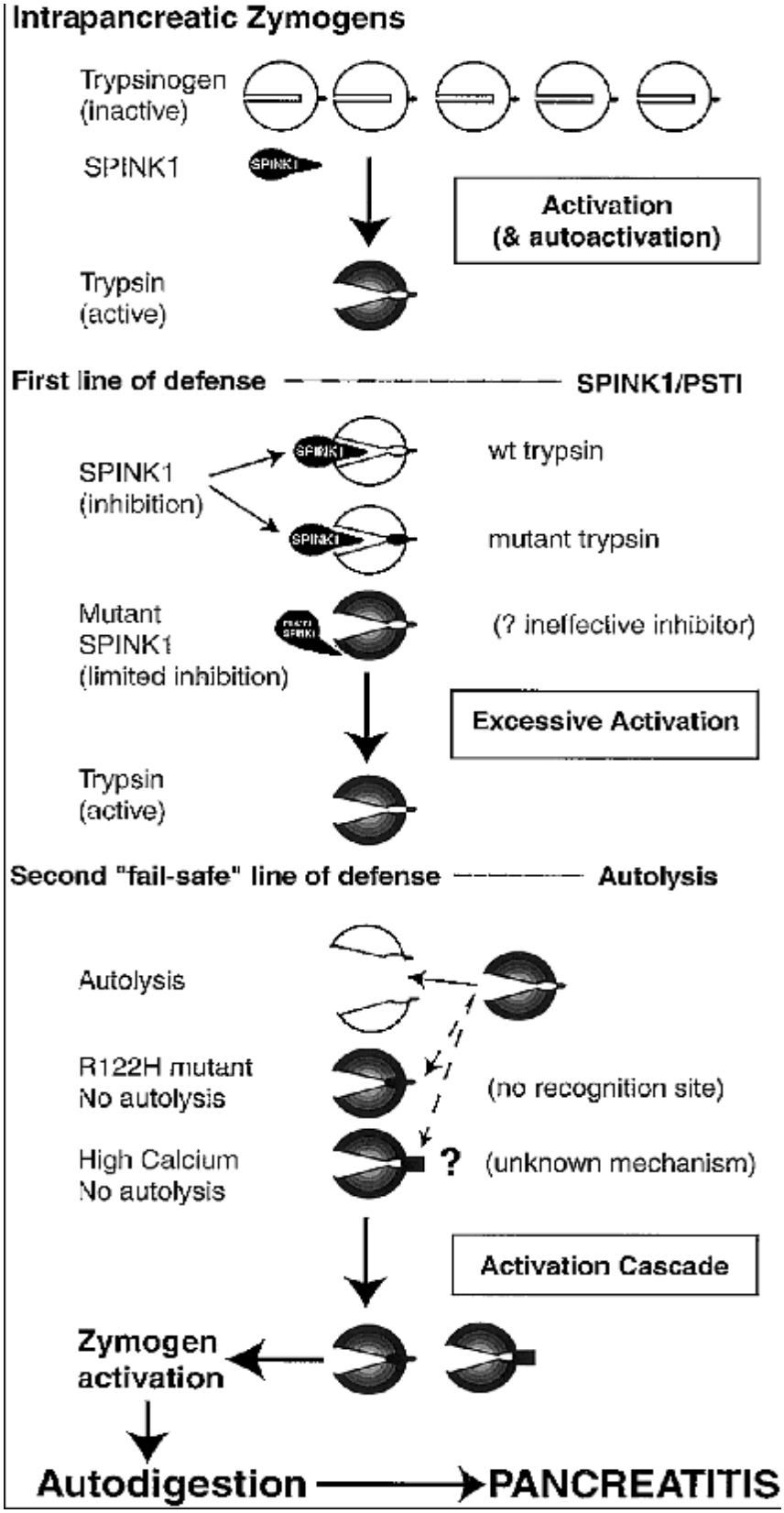
Trypsin is susceptible to rapid autolysis within the acinar cell where calcium levels are low, protected from autolysis after active secretion into the pancreatic duct and duodenum where calcium levels are high, and then undergoes autolysis in the distal small intestine after calcium is absorbed in the distal duodenum and jejunum.

Protective mechanisms against acute pancreatitis

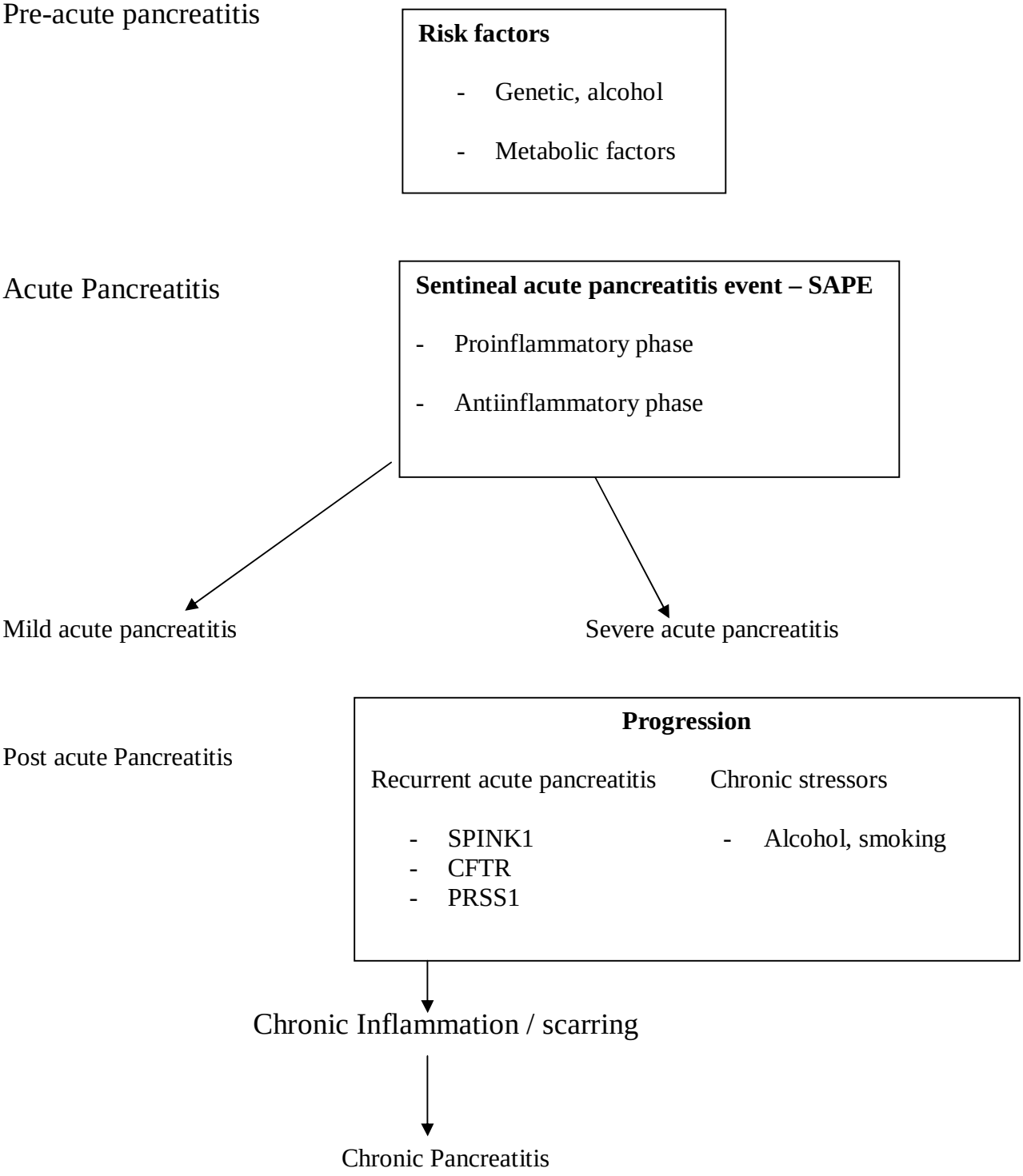
Intracellular protective mechanism include synthesis of trypsin as an inactive zymogen (trypsinogen), zymogen compartmentalization and packaging, synthesis of a specific trypsin inhibitor (PSTI or SPINK1), control of intra-acinar cell calcium levels to facilitate autolysis, and lysosome dependent pathways of zymogen / activated digestive enzyme eliminations[39].

Once the zymogens are secreted into a calcium rich juice (eliminates the autolysis protective mechanism) the pancreas is dependent on SPINK1 (inhibit prematurely activated trypsinogen) and rapid flushing of the pancreatic duct by fluid from the duct cells to protect against pancreatitis. Disruption of any of these protective mechanisms increase susceptibility to acute pancreatitis and predisposes of chronic pancreatitis. The combined effect of environment and genetic factors in increasing the risk of recurrent acute and chronic pancreatitis[39].

SPINK 1 Defense



Progression from pre-acute pancreatitis to chronic pancreatitis[40].



RECENT CONCEPTS

Two important concepts have emerged as being important in the pathogenesis of CP including the so-called TCP. These include oxidative stress and genetic mutations.

Oxidative Stress

Oxidative stress (OS) has been implicated recently in the pathophysiology of CP[41-42]. Xenobiotics are detoxified in the body through phase I and phase II pathways chiefly in the liver[43]. Increased exposure to alcohol, nicotine, petrochemical fumes may overwhelm the capacity of phase I and phase II detoxification pathways and result in oxidative stress. OS will damage these cell either directly by cell membrane destruction, depleting the cells of antioxidants or free radical mediated injury[44-46].

Genetic Mutation:

The role of genetic mutations in CP has been studied for more than 30 years. Initial studies were directed toward the association of HLA genes with pancreatitis. In 1950s, Comfort et al[47] described Heridatary Pancreatitis as a highly penetrant, autosomal dominant condition. In 1998, studies showed an increased incidence of CFTR gene mutations in patients who had idiopathic CP[48,49]. In a landmark study, Whitcomb et.all[50]. reported arginine to histidine substitution at residue 117 (subsequently renamed as R122h) in the cationic trypsinogen gene on the long arm of chromosome 7 (7q35) in hereditary pancreatitis. Witt and colleagues said that mutations in the SPINK 1 gene were associated with CP[51]. The discovery of heterozygotes individuals with mutations in multiple genes and the resultant additive effect on disease underscores the complex nature of genotype and phenotype expression in CP

Cause of chronic pancreatitis and recognized associated genetic mutations

Cause	Genetic mutation (may have 1 or more)
Alcoholic	SPINK 1 CFTR
Tropical Hereditary	SPINK 1 PRSS 1 SPINK 1 CFTR
Idiopathic	SPINK 1 CFTR

Genetic Predisposition

A genetic predisposition to chronic pancreatitis to among some families was recognised by Comfort et. al[52]. By genetic analysis it was discovered that mutation like (N291 and R122H) in the cationic trypsynogen (PRSSI) were associated with CP. Mutation in SPINK 1 have been associated with ICP and TCP. These discoveries not only provide insights into the molecular mechanism but present the possibility of powerful diagnostic tools. There are severe reasons why molecular and genetic analysis will become important in future.

1. Mutation will provide information on risk of developing pancreatitis.
2. Mutation detection will assist in early diagnosis

3. Mutation identified will provide rationale classification.
4. Molecular classification will help in knowing disease progression and prognosis.
5. Specific mutation will help in gene environmental interaction
6. Mutation may help in developing new therapeutic intervention
7. Important for patients who are seeking answers for why they have pancreatitis

Serine Protease inhibitor Kazal Type (1 (SPINK 1)

The SPINK1 is also known as pancreatic secretory Trypsin inhibitors located on chromosome 5. It is a 56 amino acid peptide that inhibits Trypsin by Physically blocking the active site[53,54]. SPINK1 is synthesised by pancreatic acinar cells. It provides the first line defense against premature Trypsinogen activation within pancreas, because it is capable of inhibiting about 20% of Trypsin activity by competitively blocking the active site of Trypsin. In 2000, the role of SPINK 1 mutation in chronic pancreatitis emerged. The most frequent mutation in (SPINK1) gene exon 3 results in asparagine to serine aminoacid change (N34 S) which leads to decreased trypsin inhibitory capacity. SPINK 1 (N34S), mutation are relatively common seen in 2% of general population. SPINK 1 mutation in patients with idiopathic chronic pancreatitis is markedly increased Proving that they are associated with chronic pancreatitis.Witt and colleagues were first to study an association between SPINK and idiopathic Chronic pancreatitis chronic pancreatitis have occurred with homozygous, heterozygous genotypes. SPINK 1 mutation appears to act on disease modifer lowering the threshold for initiating pancreatitis and hence important role in recurrent acute pancreatitis and chronic pancreatitis[55,56]. The severity

of pancreatitis appears to be similar between homozygous, heterozygous or compound heterozygous genotypes suggesting that genetics is complex.

Cationic Trypsinogen mutations

The Cationic Trypsinogen mutation is seen in many cases of hereditary pancreatitis. The R122H and N29I mutations interfere with autolysis and cause premature trypsinogen activation[5,39,57]. Nearly 60 – 80% who inherits the mutation will develop pancreatitis. Nearly 50% of individuals with acute pancreatitis will develop chronic pancreatitis.

CFTR[39]

CFTR gene is located on the long arm of chromosome 7, when both alleles of CFTR gene are involved; cystic fibrosis with pancreatic insufficiency can develop. Compound heterozygosity of CFTR gene with one severely affected and one moderately affected allele has shown to be cause of recurrent pancreatitis. CFTR may be part of a complex process in which heterozygous CFTR and heterozygous SPINK 1 mutations cause recurrent acute pancreatitis.

Cathepsin B: (CTSB)

Cathepsin B is a lysosomal cysteine proteinase involved in the initial activation of Trypsinogen[58]. Recent study by Madhurkar et al showed CTSB mutation doubled the risk for Tropical Pancreatitis[59].

Idiopathic chronic pancreatitis[60]

Idiopathic chronic pancreatitis includes patients in whom no associated factors can be identified. The discovery of new genetic factors, environmental factors and metabolic factors will reclassify - and reduce the numbers of patients in this category. Idiopathic chronic pancreatitis has long been shrouded in mystery as far as its etiopathogenesis is concerned. Many different theories have been proposed like immune-mediated injury and environmental toxins however newer studies have discarded above hypothesis. Idiopathic CP that is prevalent in India is also known as tropical calcific pancreatitis by some authors[61].

Early and late onset

The age of onset in idiopathic pancreatitis is bimodal as observed by Layer et al. In early onset calcification and exocrine and endocrine deficiency develops slowly than late onset. In late onset pain was absent in nearly 50% of patients. Pbtizone[62] indentified SPINK 1 mutation in about 25% of patients with idiopathic chronic pancreatitis. Patients with SPINK1 mutation developed pancreatitis before age of 20 in many studies.

Tropical Chronic Pancreatitis[17]

Tropical chronic pancreatitis is referred to as type of idiopathic CP occurring in tropical countries. They can be subgrouped as Tropical calcific pancreatitis. TCP Characterised by multiple episodes of abdominal pain in childhood, extensive pancreatic calcification and pancreatic dysfunction but no diabetes at time of diagnosis. The other group is called as fibrocalculous pancreatitis diabetes (FCPP) in which diabetes mellitus is the first major complaint[63]. The etiology of tropical calcific pancreatitis is poorly understood. Many theories environmental factors, malnutrition,

dietary Toxin like cyanogenic glycosides and micronutrient deficiency have been proposed. Recent reports from genetics studies have shown significant association between (SPINK1 and cationic trypsinogen mutation)

CLINICAL FEATURES

Recurrent acute Pancreatitis[64]

All patients have recurrent attacks of upper abdominal pain at the onset. The pain is accompanied by nausea and vomiting in around 90% of patients. Patients can also present with severe pancreatitis which is associated with organ failure.

Chronic Pancreatitis

The abdominal pain is the common presenting symptoms. The patient experience many attacks of severe pain in the middle or upper abdomen. The natural history of pain is highly variable and can present as intermittent or chronic pain. The other symptoms include diarrhea, weight loss, endocrine insufficiency (diabetes), jaundice or complications of acute episodes. The physical examination does not help to establish diagnosis however fullness, tenderness can be elicted. Patients with advanced disease show signs of malnutrition

Approach to Determine etiology of recurrent Pancreatitis:

Evaluation of a patient after the first attack of pancreatitis includes a careful history, lab studies (lipid profile, serum calcium, Anti nuclear anti body, Liver function tests) and USG abdomen[65,66]. Approximately 20% to 50% of patients with acute pancreatitis will have a recurrence. EUS / MRCP should be done to identify gall bladder sludge, small CBD stones, pancreas divisum, pancreatic tumors and early chronic pancreatitis. In clinical practice, ERCP is seldom done. For patients with

idiopathic acute pancreatitis, selective use of genetic testing may be appropriate. For the patient with RAP, especially in the setting of an appropriate family history, genetic testing for cationic trypsinogen gene (PRSS1), SPINK 1 gene can be done. The role of genetic testing for these mutations is less clear and controversial[57].

Chronic Pancreatitis:

The diagnostic approach to chronic pancreatitis has evolved considerably in recent years. The investigations used can be summarized as below:

- Imaging of pancreas and pancreatic ducts.
- Tests for pancreatic exocrine insufficiency: to assess the degree of exocrine dysfunction (e.g.,fecal chymotrypsin) and sometimes to monitor replacement therapy(e.g.,fecal fat estimation).
- Tests for pancreatic endocrine deficiency.

Biochemical Blood Tests

Serum trypsin exists mainly as the cationic form of its precursor trypsinogen. In states of pancreatic insufficiency, the precursor gets converted to active trypsin. The active trypsin is mostly bound by protein inhibitors and become undetectable by catalytic assays[69]. The study by pezzilli[70] et all has found it to be 28% sensitive but 100% specific in identifying chronic pancreatitis. However, other studies have shown it to be only 50-60% sensitive in detecting chronic pancreatitis. Serum lipase and amylase levels are elevated during acute pancreatitis but are of limited value to diagnose chronic pancreatitis.

IMAGING TESTS:

Transabdominal Ultrasonography

Transabdominal ultrasonography can be used to visualize alterations of the pancreatic duct, pancreatic calcification or stones. Abdominal ultrasound is highly sensitive in detecting severe chronic pancreatitis but the sensitive is much less in milder forms of the disease[71].

Computed Tomography (CT) Scan[72]

Computed tomography scan is one of the important diagnostic modalities in detection of chronic pancreatitis. In chronic pancreatitis, a non-contrast-enhanced CT scan shows chronic pancreatitis. The contrast-enhanced pancreatic imaging produced by thin-multidetector row scanners can detect any abnormality in the size and shape of the gland, any parenchymal attenuation, any dilation or stones in the pancreatic duct and pancreatic pseudocysts.

Magnetic Resonance Imaging (MRI)[73]

Magnetic resonance imaging cannot detect extraductal pancreatic calcification as well as **CT sacn** can. Both **T1and T2** weighed images may be used to detect severe forms of chronic pancreatitis, though the findings are less specific in the elderly patients.

Endoscopic Retrograde Cholangiopancreatography(ERCP)

ERCP can be used as a diagnostic as well as therapeutic tool in patients with chronic pancreatitis. It can detect dilatation and stenosis of pancreatic ducts, pancreatic stones or cysts.

Endoscopic Ultrasonography (EUS)[74]

Endoscopic ultrasound is the diagnostic modality of choice to evaluate patients with early or mild chronic pancreatitis. EUS helps enables the clinician to assess the pancreatic parenchyma as well as the pancreatic duct. The ducts are studied for narrowing, dilation, irregularity, calculi, side-branch dilation and hyperechoic walls EUS is considered superior to ERCP or MRCP for detection of early or mild changes of chronic pancreatitis as well as small duct involvement.

GENETIC TESTING

Genetic testing, though not a preferred diagnostic tool directly involved in detection of chronic pancreatitis, can be done in with suspected hereditary or idiopathic disease. The test is not routinely available and the screening test too expensive for routine usage.

TREATMENT

Recurrent Acute Pancreatitis

Mild pancreatitis is treated with supportive care including pain control, intravenous fluids. Severe pancreatitis requires intensive care and monitoring and advanced fluid resuscitation. The role of antibiotics have be debatable. Nutritional support is required for severe pancreatitis with parenteral or enterally.

Chronic Pancreatitis

Management options for chronic pancreatitis include medical, endoscopic and surgical treatments. Patients with chronic pancreatitis seek medical attention because they suffer from abdominal pain, weight loss or diabetes.

Treatment of Pain[75,76]

The pathophysiology of pain in chronic pancreatitis is incompletely understood. The proposed theories are ductal and mechanical mechanism, neuropathic, oxidative stress and central mechanism of pain. The management of pain in chronic pancreatitis is frustrating both for patients and clinicians. The following tables summarize the management of chronic pancreatitis.

Mechanisms of Pain in Chronic Pancreatitis and Management Options[77]

Proposed mechanism of pain	Management options
Duodenal Obstruction	Surgical bypass or endoscopic stent
Bile duct obstruction	Endoscopic stent or surgery
Pseudocyst	Endoscopic, surgical or percutaneous drainage
Pancreatic duct obstruction (stone or stricture)	Endoscopic or surgical ductal decompression
Tissue hypertension and ischemia	Antioxidants, endoscopic and surgical ductal decompression
Intra-pancreatic nerve injury	Celiac plexus block or neurolysis
Visceral nerve sensitization	Tricyclic antidepressants, SSRI, combined serotonin and norepinephrine re-uptake inhibitors
Central nerve sensitization	Tricyclic antidepressants, SSRI, combined serotonin and norepinephrine re-uptake inhibitors
Elevations in cholecystokinin	Non-enteric coated pancreatic enzymes

Options for Medical Management of Pain in Chronic Pancreatitis[75,76]

Agent	Dose
Propoxyphene with acetaminophen	1-2 po q8h
Tramadol (50 mg)	1-2 po q8h
Antioxidants	A combination of 500 – 1000 mg of Vit C, 250-300 IU of Vit E, 500 – 800 ug of selenium, 2 g of methionine, 9000 – 10,000 IU of beta carotene per day in divided doses
Tricyclic antidepressants	Amitriptyline (start at 25 mg qhs)
Pancreatic enzymes	Non enteric coated, protease content 25,000 – 50,000 USP with each meal Co-treatment with H2 blockers and PPI if needed to prevent degradation by gastric acid

MATERIALS AND METHODS

Study Design

Prospective analysis of patients with idiopathic recurrent acute and idiopathic chronic pancreatitis attending PSG Hospital, Coimbatore during the period Novermber 2010 to December 2011.

Study centre

The study was done in the Department of Gastroenterology PSG Institute of Medical Sciences and Research (PSG IMSR) in collaboration with the Centre for Molecular Medicine and Treatment (CMMT), PSG IMSR, Coimbatore.

Ethical approval:

The study protocol was approved by the Institute Human Ethics Committee (IHEC) prior to the start of the study.

Subjects:

Total of 50 patients were included in the study out of which 33 patients were idiopathic chronic pancreatitis and 17 patients were idiopathic recurrent acute pancreatitis.

Inclusion Criteria

Criteria for Idiopathic recurrent pancreatitis:

1. Two or more documented episodes of typical pancreatic type of abdominal pain
2. Amylase or lipase greater than 3 times the upper limit of normal
3. Features of acute pancreatitis on imaging studies (ultrasound / CT abdomen)
4. No identifiable cause or risk factors

Diagnosis of Idiopathic chronic pancreatitis:

The diagnosis of idiopathic chronic pancreatitis was made based on clinical setting and evidence of pancreatic duct dilatation irregularity and/ or pancreatic calcification on imaging studies without any identifiable cause or risk factors.

Work up

All patients underwent complete blood counts, biochemical investigations including liver function test, renal function, fasting blood sugar, serum calcium, lipid profile, serum amylase and lipase, antinuclear antibody, endocrine workup Ig4 levels viral serology and bile for microlithiasis after informed consent. The following imaging studies were done

1. Transabdominal ultrasonography
2. Contrast enhanced CT abdomen
3. MRCP / EUS if indicated

Complications of Chronic Pancreatitis like diabetes mellitus, steatorrhoea, bile duct obstruction, pseudocyst were diagnosed as per standard criteria either biochemically or imaging.

Exclusion Criteria:

1. Patients with identifiable cause and risk factors for chronic and recurrent pancreatitis.
2. Malignancy
3. Retroviral infection
4. Psychiatry illness
5. Hereditary and genetic diseases

Genetic analysis

Steps involved in genotype analysis were

- DNA extraction
- PCR to amplify SPINK gene
- RFLP using Pst I restriction enzyme
- Poly Acrylamide Gel Electrophoresis (PAGE) of the digested product

DNA extraction was done according the standard protocol as follows

- Blood sample 300µl + 1 volume of cell lysis buffer + 3 volume of sterile MilliQ water mixed up in 2 ml Eppendorf tubes which were then incubate on the ice 4°C for 10 minutes.
- The samples centrifuged at 4° C for 20 minutes at 4000 rpm.
- The supernatant discarded and again 150µl of same cell lysis buffer added in every sample and 480 µl of autoclaved H2O also added.
- The sample then centrifuged at 4°C for 20 minutes at 4000 rpm. Then again the supernatant was discarded.
- Then 720µl of nucleic acid lysis buffer, along with add 15µl of RNAse added to the eppendorf tube.
- The samples were vortex mixed and incubated for 10 -15 minutes at 37°C. 30µl of 10% SDS and 30 µl of Proteinase K then added.
- The sample was incubated in water bath or heat block at 55°C for 2-3 hours.
- Equal volume of phenol: chloroform (1:1) added and centrifuged at 15800rpm for 5 minutes.
- The supernatant of the samples (aqueous layer) transferred to the new tube.

- Equal volume of chloroform again added and centrifuged at 13000rpm for 5minutes.
- The supernatant then transferred to new tube and add 0.1x volume of 3M of sodium acetate (pH 6.0) and 2x volume of 100% ethanol and mixed well, centrifuged at 13000rpm for 10 minutes.
- Then DNA was completely washed with 70 % ethanol and centrifuged again at13000rpm for 10 minutes. Supernatant ethanol discarded and the tubes dried. The DNA pellet was re-suspended in sterile water or TE buffer. DNA sample stored at -20°C. Quantification of the extracted genomic DNA was done using Nanodrop quantification after 0.8% agarose gel electrophoresis.

PCR amplification of SPINK gene

1. Primers

Forward primer SPINKF:TTCTGTTTAATTCCATTTTTAGGCCAAATGCTGCA

Reverse primer SPINKR: GGCTTTTATCATACAAGTGACTTCT

2. DNTPs (Himedia)
3. Taq DNA polymerase (colourless Taq Genei)
4. Taq Buffer A (Genei)
5. DNA Sample Optimised concentration: 0.05µg
6. Milliq water

Table 1: PCR Reaction Mix- Volume of reagents used

S. NO	COMPONENTS	VOLUME	FINAL CON
1	Forward primers - 1 µM	1 µL	50 nM
2	Reverse primers - 1 µM	1 µL	50 nM
3	Buffer A	2 µL	1.5mM $Mgcl_2$
4	DNTPs	0.8 µL	100µM each DNTPS
5	Taq enzyme	0.2 µL	0.01 unit
6	DNA sample (0.15µg)	-	0.15µg/ 20µL
7	Milli Q Water	UPTO 20 µL	

PCR reactions were performed on Eppendorf thermocycler.

The mix was kept in cycler with following conditions.

PCR PROGRAME

1. **Initial denaturation - 94°C for 10 min**
2. **Denaturation - 94°C for 50 sec**
3. **Annealing - 57.7°C for 50 sec** } **40 CYCLES**
4. **Extension - 72°C for 1 min**
5. **Final extension - 72°C for 5 min**
6. **Then held at 4°C**

At the end of reactions, PCR amplification was confirmed by electrophoresis on 2% agarose gel.

2% Agarose gel electrophoresis

1. To 30 ml of 1X TAE buffer, 600 mg of agarose was added and the contents were heated in
2. a microwave until it formed a clear solution.
3. To this 0.2µl of ethidium bromide was added and the solution was poured into a trough with
4. a comb
5. The solution was allowed to set for approximately 30 minutes.
6. Once set, the comb and the tape around the trough are removed.
7. The trough was then placed in an electrophoresis tank containing 1X TAE buffer. The
8. trough should just immerse in the buffer.
9. Loading dye was mixed with 100bp ladder in a sterile PCR tube.

10. 15 µ of each PCR DNA with 2µl of dye aliquotted separately in PCR tubes.
11. The wells were loaded and electrodes were connected and run at 80V for 45 minutes until
12. the loading dye was seen for 3/4th of the gel.
13. The trough was removed and gel viewed under UV illuminator for the presence of bands of product size **320bp**

Quantification of PCR DNA Product using Nanodrop quantification.

Spectrophotometer was used to check the quality and quantity of the extracted DNA. The Principle here involves measuring optical density of the DNA sample at 260 nm and 280 nm Wavelengths. The purines and pyrimidines in DNA absorb UV radiation at 260 nm and the aromatic aminoacids in proteins absorb UV radiation at 280 nm. The ratio of optical density at 260 and 280 nm is an estimate of the DNA quality. The optimal range for DNA of high Quality is 1.8 to 2.0. The concentration of the DNA sample was also determined Spectrophotometrically.

RFLP Enzyme Digestion

Requirements

1. PstI (Fermentas 10 units/ µL)
2. 10 x Buffer O
3. PCR product (optimized concentration 0.6 µg)
4. 37°C incubator

The PCR amplified product was digested with PstI restriction enzyme at 37°C overnight. The 320 base pair (bp) DNA fragment was split into 286 and 34bp DNA Fragments if A→G substitution of the SPINK gene is present.

Table 2: Reaction Mix for RFLP

S. NO	COMPONENTS	VOLUME
1	Pst I Enzyme	1.0 µl
2	10X Buffer	2 µl
3	PCR product Concentration	0.6 µg
4	Milli Q Water	Upto 31.5 µl

Reaction mix was incubated overnight at 37^0C

- Restriction digestion product was inactivated by adding 1.3 µl of 0.5 M EDTA before gel Electrophoresis.

Polyacrylamide Gel Electrophoresis

The DNA Fragments were separated using 12 % Polyacrylamide gel electrophoresis Polyacylamide gel prepared from the following reaction mix.

Table 3: Reagents to prepare 12% Polyacrylamide gel

12%	5mL	10mL	15mL	20mL	25mL	30mL	40mL	50mL
Water	1.75	3.4	5.15	6.8	8.55	10.1	13.6	16.9
A:B (30:0.8)	2	4	6	8	10	12	14	20
1.5M Tris pH 8.8	1.3	2.5	3.8	5	6.3	7.5	10	12.5
10% APS	0.05	0.10	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

- Polyacrylamide gel was run in Amersham electrophoresis system at 90 V for about 7 hours.
- The gel was then stained with ethidium bromide
- The stained gel was viewed in a Chemiluminescence gel documentation system to
- Identify the DNA fragments

Identification of Genotypes

The genotypes were determined based on the expected product size.

A/A Genotype (Asn 34 Asn) - 320bp

G/G Genotype (Ser 34 Ser) - 286bp

A/G Genotype (Asn 34 Ser) - 320,286 and 34

Statistical Analysis

Data were collected and analysed and expressed as mean and standard deviation. The SPSS version 10.0 program was used for statistical analysis.

RESULTS

Recurrent Acute Pancreatitis (RAP)

There was total of 17 patients and mean age of patient was 22.29± 9.70 years and the youngest being 14 years and the oldest 39 years with 14 patients below 30 years of age. The duration of illness was 28.23±10.34 months and mean fasting glucose level was 91.64±15.94 mg /dl. The following graph represents age category

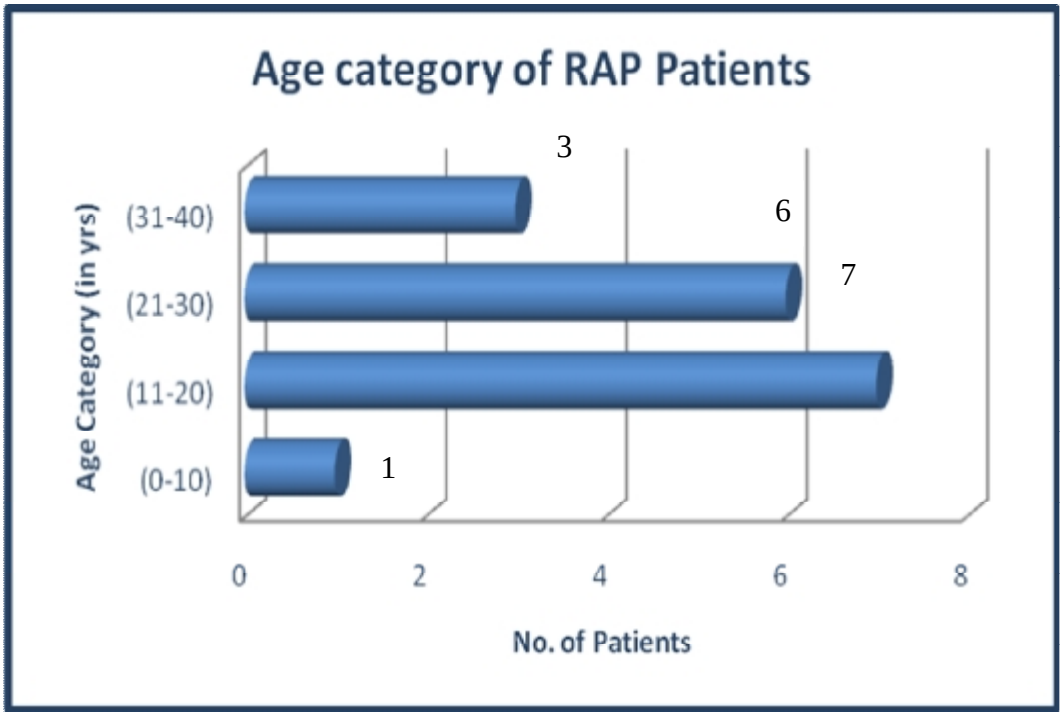


Figure 1

Sex Distribution:

82% of patients were males and 18% were females suggesting male preponderance

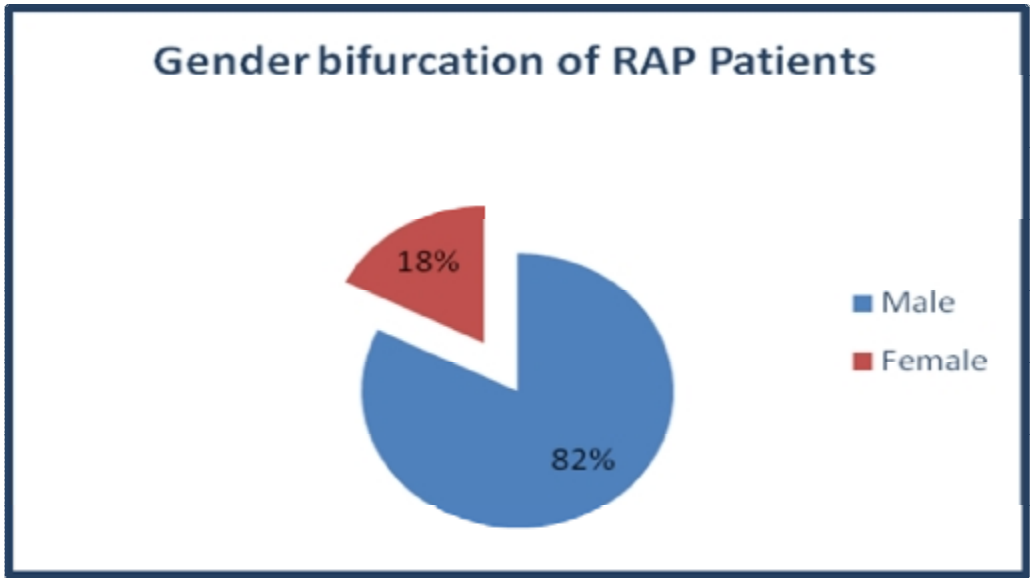


Figure 2

Number of Episodes:

The number of episodes of recurrent acute pancreatitis in patients is shown in the Table 1. The majority of the patients had 3 episodes. Patients with spink positive had more number of episodes compared to wild type. All the SPINK positive patients had 4 and above episodes.

Episodes	No. of Patients	No. of SPINK positive
Upto 3	8	-
3 -4	2	5
4-5	-	1
> 5	-	1

Table 1

Body Mass Index

Majority of patients had BMI >18.5

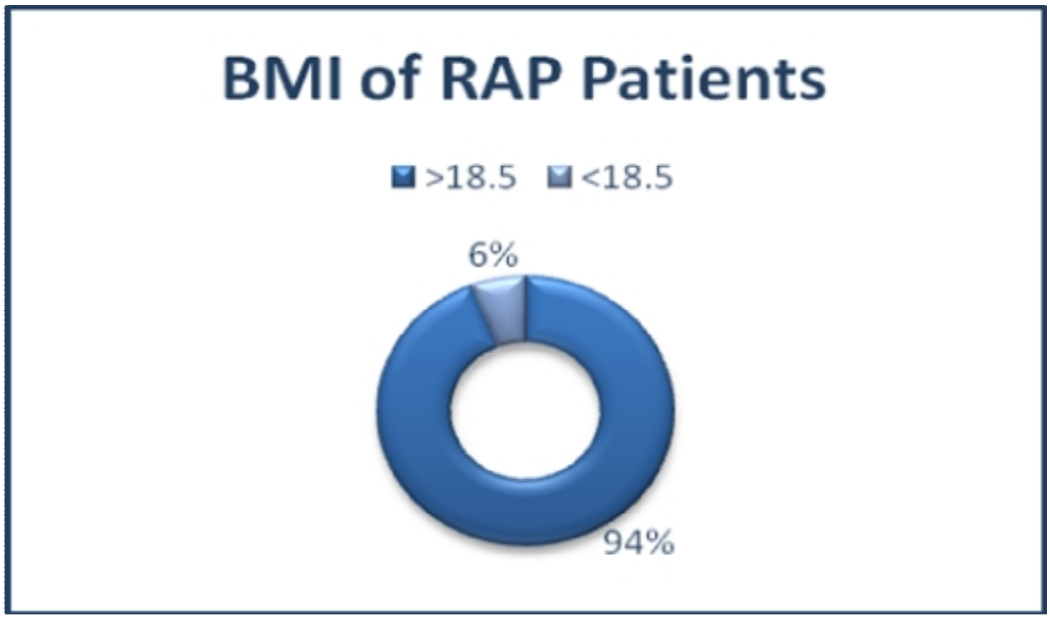


Figure 3

SPINK Mutation

Out of 17 patients with idiopathic recurrent pancreatitis 41.17% (7) were positive for mutation

Spink Mutation	No. of Patients
Wild Type	10
Hetrozygous	7

Table 2

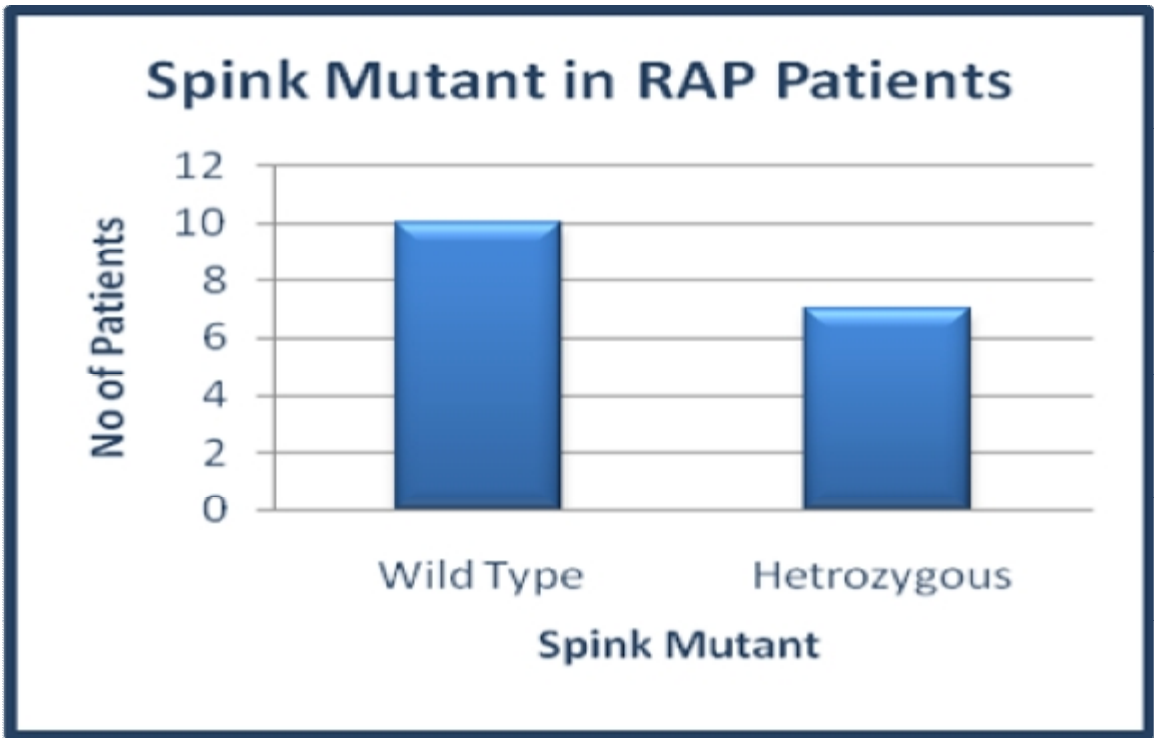


Figure 4

Age category and BMI of SPINK mutant patients

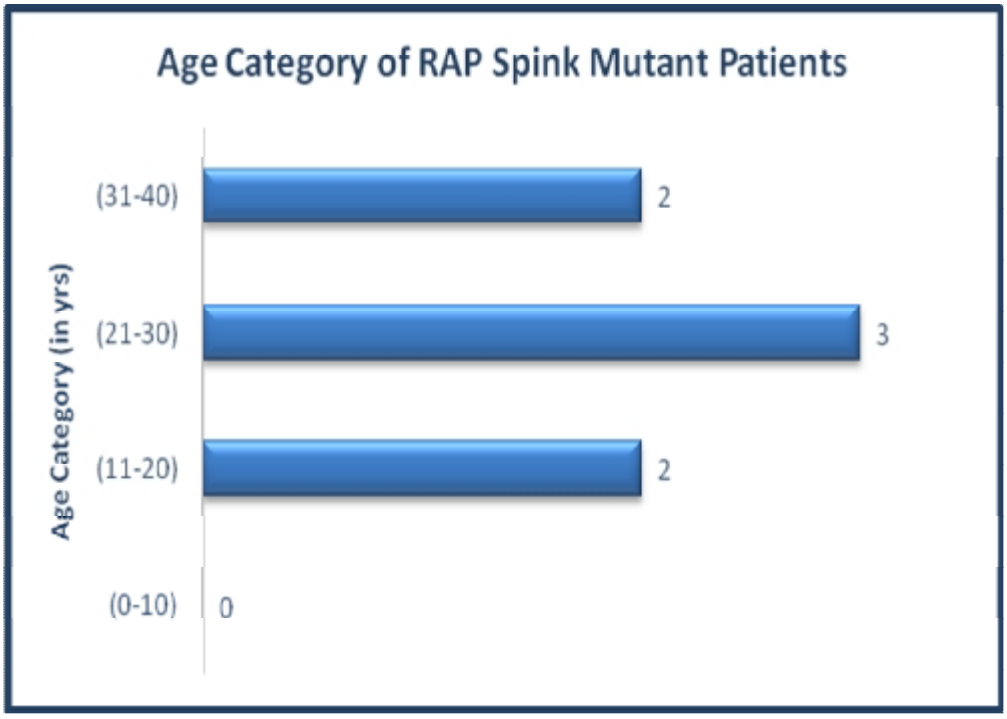


Figure 5

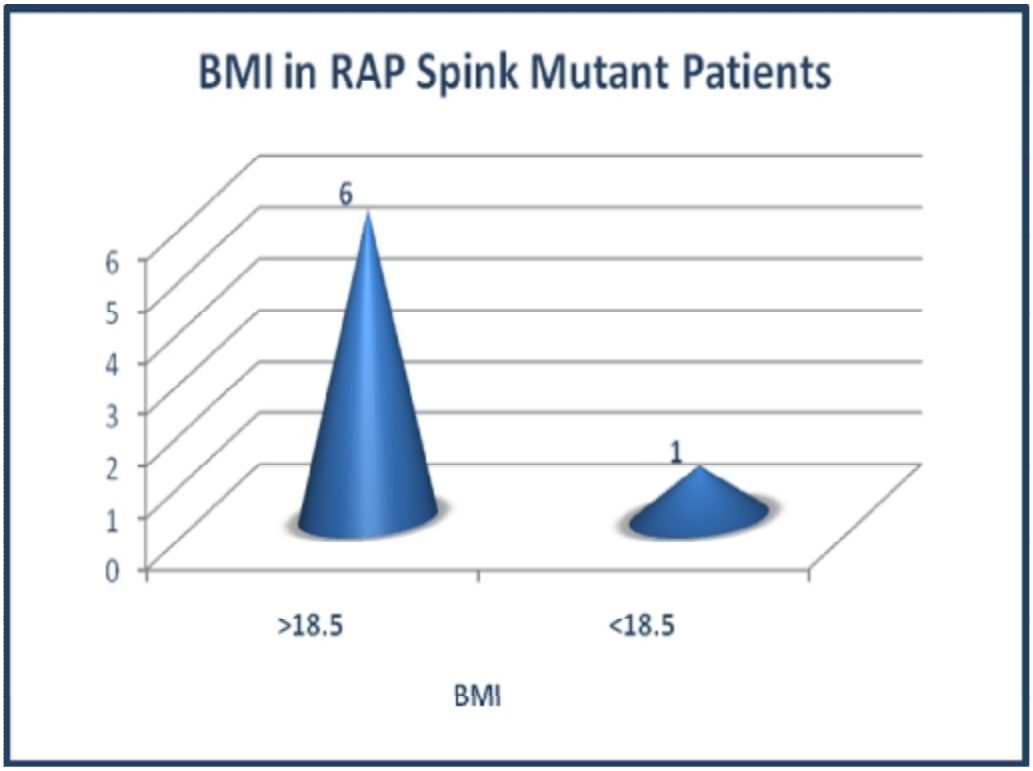


Figure 6

IDIOPATHIC CHRONIC PANCREATITIS

There were 33 patients included and mean age was 31.75±13.07 years with youngest being 9 yrs and oldest being 69yrs. The mean age of patients with SPINK positive was 31.96yrs. The mean duration of illness was 31.33±19.89 months and mean fasting sugar level was 112.57mg /dl

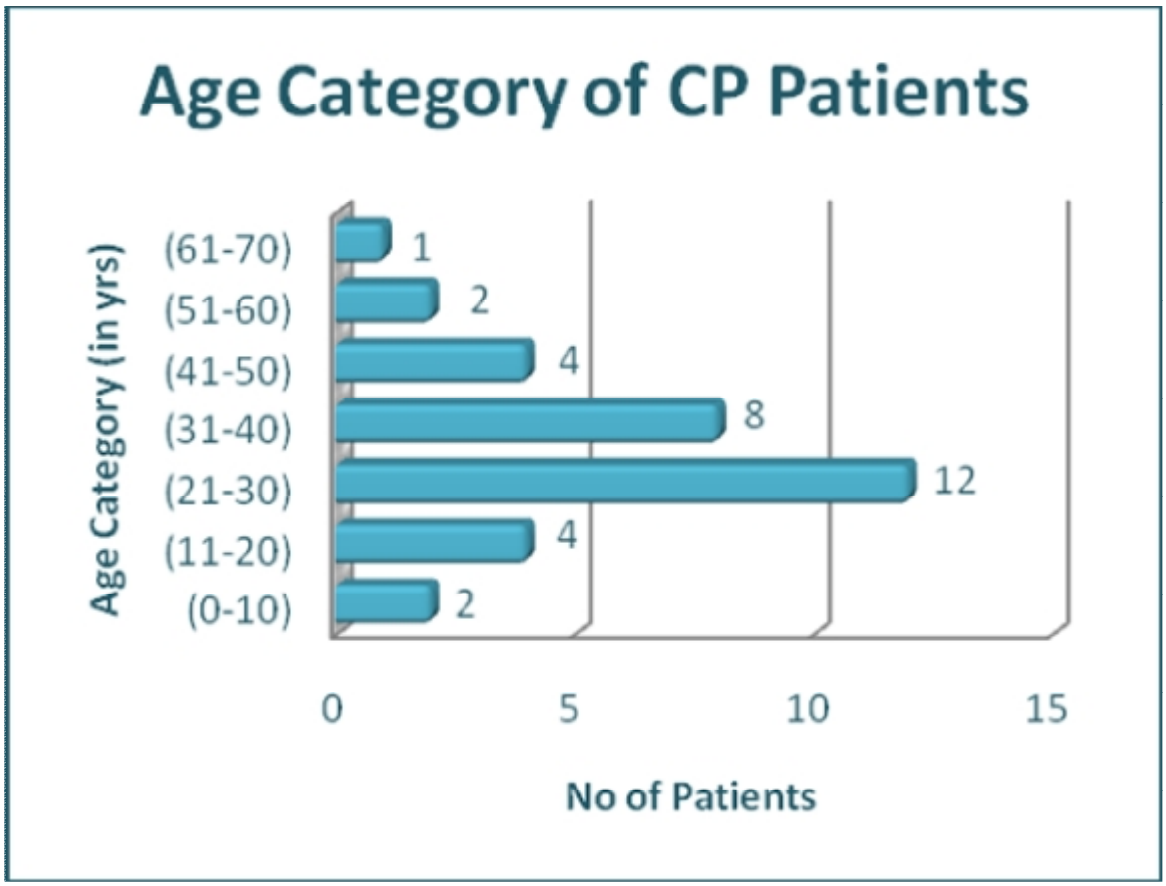


Figure 7

Gender Bifurcation

67% of patients were males and 33% were females.

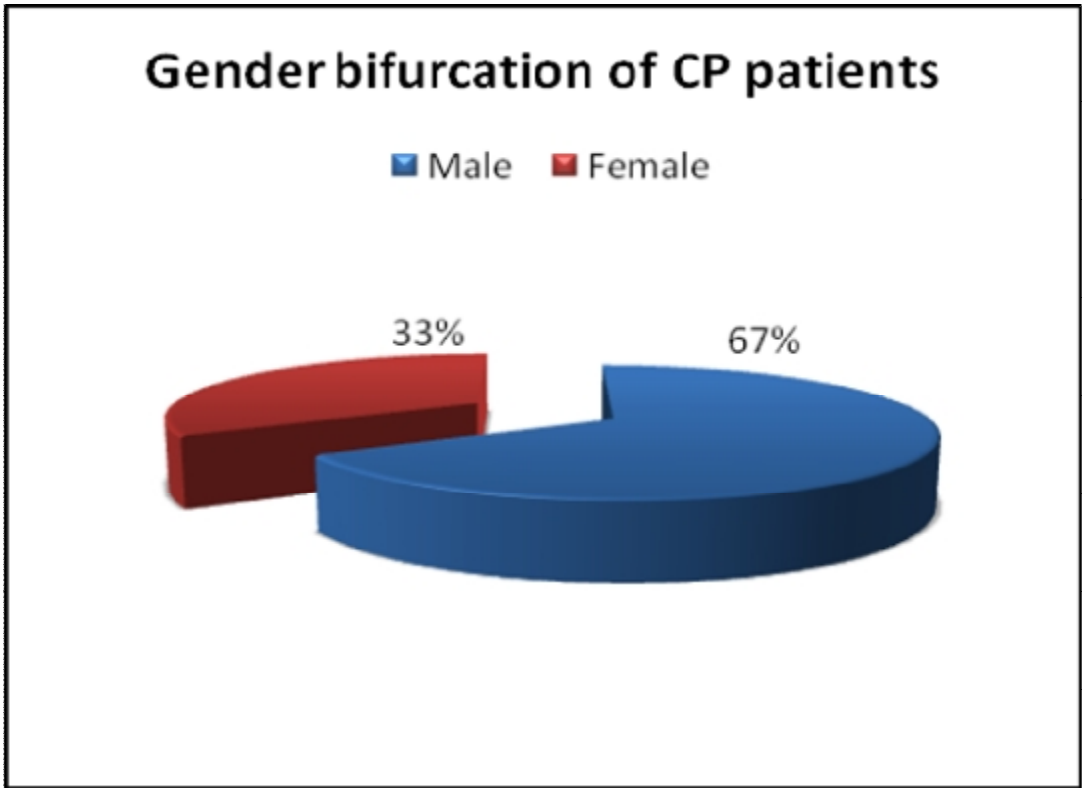


Figure 8

Clinical Features

Nearly 93.34%of patients had pain as their clinical symptoms. The following table shows various manifestations among 33 idiopathic chronic patients.

Clinical Features	No. of Patients N = 33
Pain	31 (93.94%)
Weight Loss	8 (24.24%)
Diabetes	11 (33.33%)
Steatorhea	5 (15.15%)
Bile Duct Structure	2 (6.06%)
Pseudocyst	2 (6.06%)
Jaundice	2 (6.06%)

Table 3

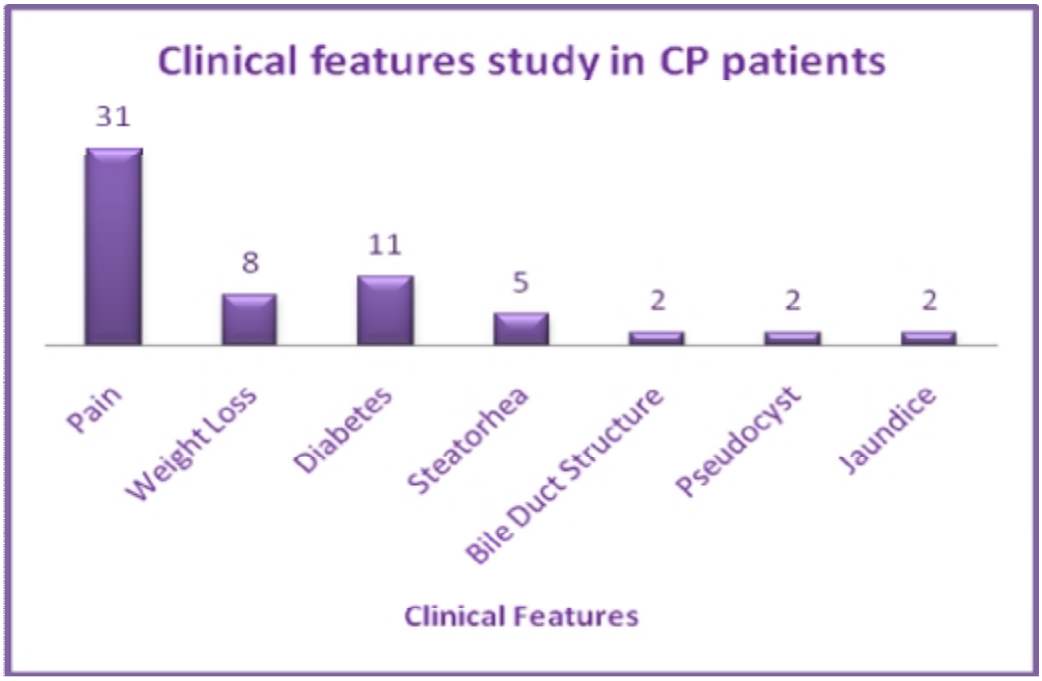


Figure 9

Body mass index

Nearly 78.78% of patients had no evidence of malnutrition as evidenced by BMI >18.5

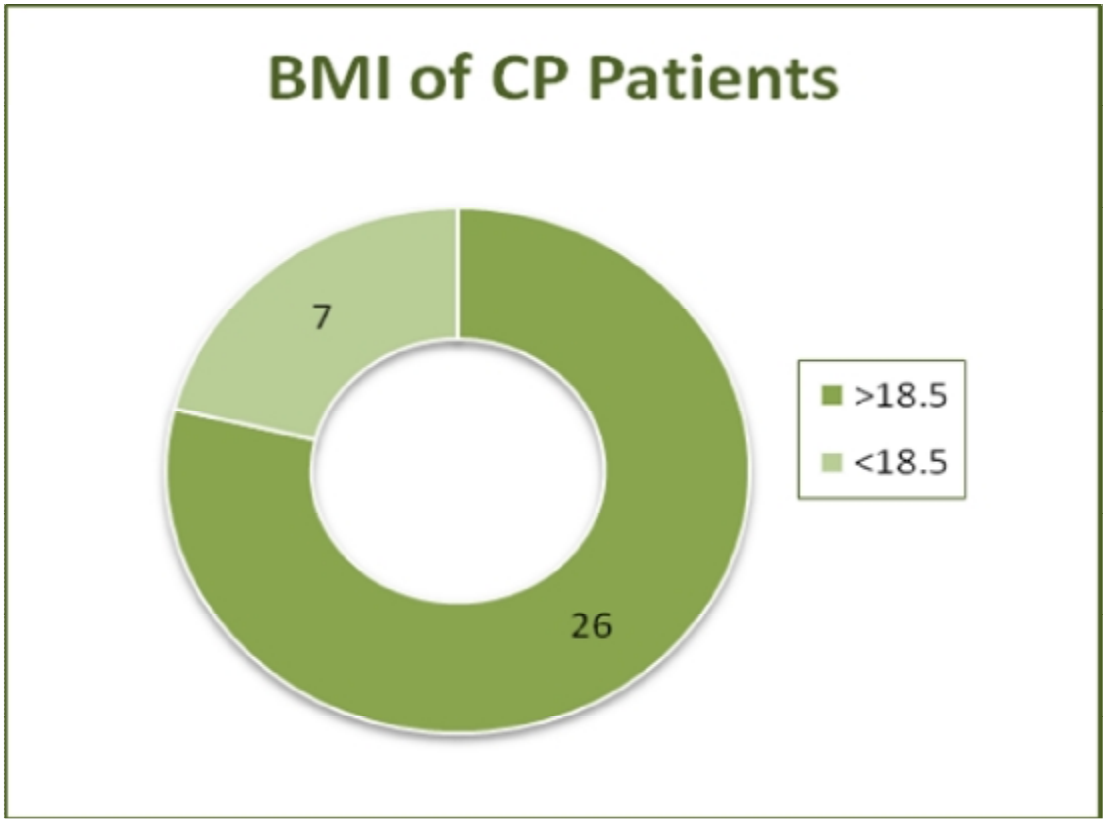


Figure 10

CT Findings

73.75% (25) of patients had ductal dilation and around 87.87% (29) had parenchymal calcification. All the patients with SPINK positive had 100% parenchymal calcification. The following table represents CT findings among 33 patients

CT Findings	No. of Patients N=33	Spink positive N=12
Ductal Dilation	25	10
Parenchymal Calcification	29	12
Ductal Calculi	7	2
Atrophy Pancreas	21	9

Table 4

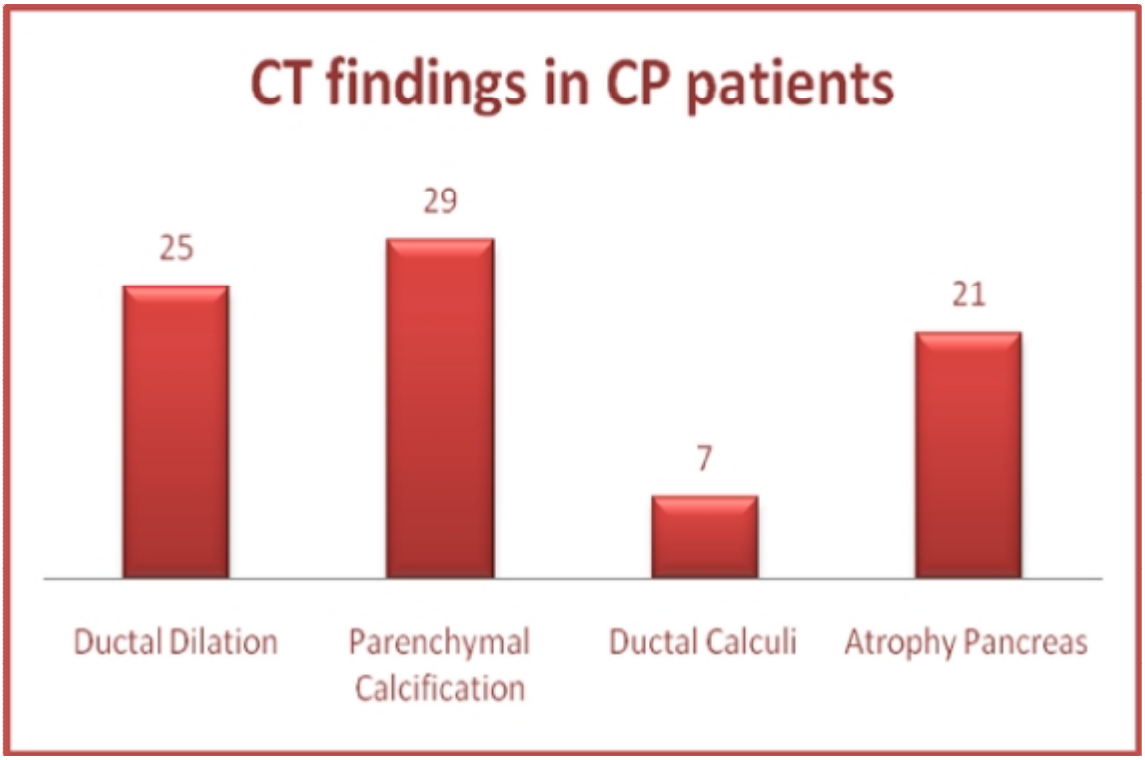


Figure 11

SPINK Mutation

36.36% of patients were having spink mutation. The following graph represents the number of patients with age category of spink mutation.

Spink Mutation	No. of Patients
Wild Type	21
Hetrozygous	10
Mutant	2

Table 5

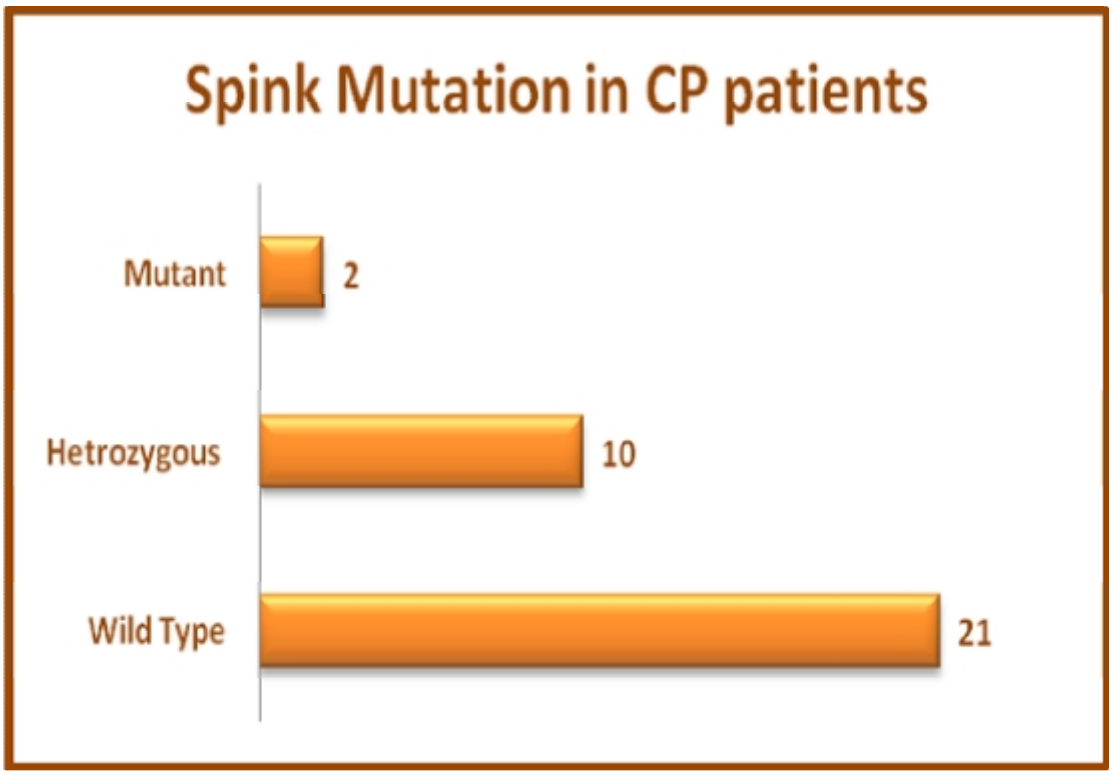


Figure 12

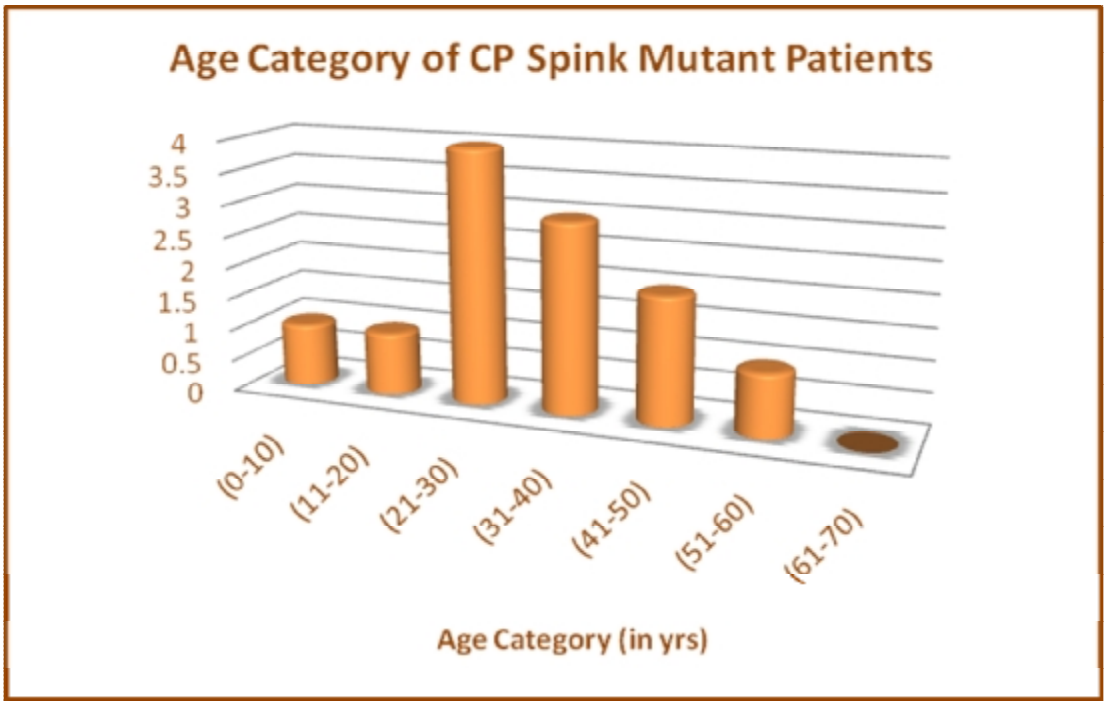
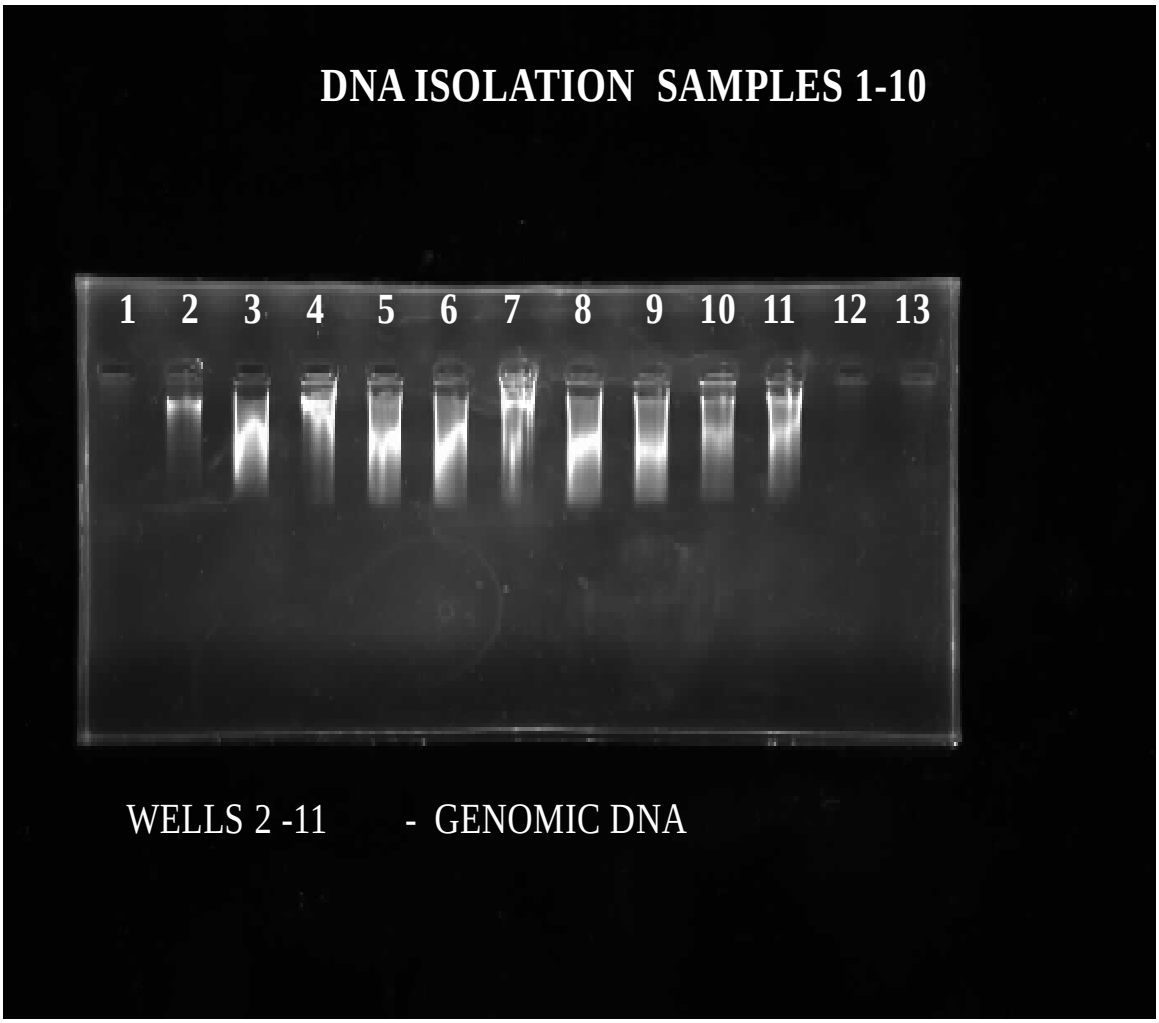


Figure 13

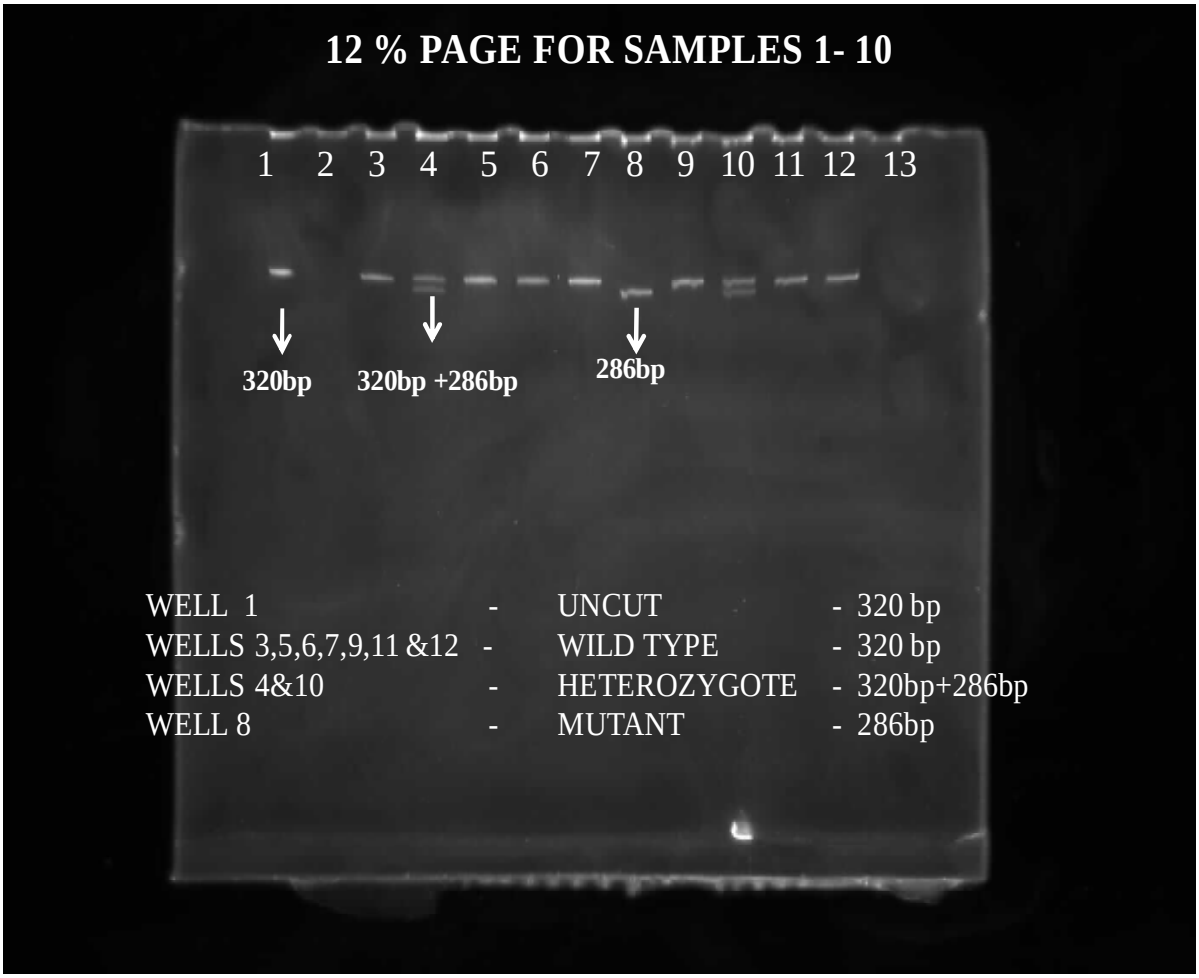
BMI in SPINK mutant patients

BMI	Spink Mutant Patients
>18.5	11
<18.5	1


DNA ISOLATION SAMPLES 1-10
1 2 3 4 5 6 7 8 9 10 11 12 13
WELLS 2 -11 - GENOMIC DNA

RFLP PCR FOR SAMPLES 1-10

WELLS 1 -5 , 7-11 - PCR PRODUCT - 320bp
WELL 6 - REFERENCE PCR PRODUCT - 215bP


12 % PAGE FOR SAMPLES 1- 10
1 2 3 4 5 6 7 8 9 10 11 12 13
320bp
320bp +286bp
286bp
WELL 1 - UNCUT - 320 bp
WELLS 3,5,6,7,9,11 &12 - WILD TYPE - 320 bp
WELLS 4&10 - HETEROZYGOTE - 320bp+286bp
WELL 8 - MUTANT - 286bp

DISCUSSION

The current study is a prospective analysis of 17 patients diagnosed to have idiopathic recurrent acute pancreatitis and 33 patients of idiopathic chronic pancreatitis. Idiopathic chronic pancreatitis has been increasing in India and clinical profile is different compared to Tropical calcific pancreatitis.

Recurrent Acute Pancreatitis (RAP)

The mean age of patients in our study was 22.29 years. The mean age of patients in the studies done by Wang etal[14] and Gullo[15] et al were 41 and 43 years .The slightly older age of presentation in the above studies was due to alcohol etiology .All our patients had recurrent episodes of more than two which was similar to the study by Garg[78] et al . Our study had 17 patients out of which 7 (41.17%) were positive for SPINK mutation and all 7 patients had more no of recurrent episodes compared to wild type (no mutation). The study by Whitcomb[30] has shown that significant cases of idiopathic recurrent acute pancreatitis have genetic components like SPINK, CFTR. His genetic theory on pancreatitis says that carrying SPINK mutation leads to increased episodes of acute pancreatitis and which leads to chronic pancreatitis later .Giulia[79] et al however did not find a statistically significant association with this mutation.

Idiopathic Chronic Pancreatitis (ICP)

The mean age of ICP was in our study was 31.75 years. A study by Balakrishnan[80] et al showed mean age of patients was 30 years. Data from Layer et al[81] from United States should mean age of 19 yrs. Kandula et al[82] showed idiopathic chronic pancreatitis occurred among children and adolescents. The mean age of patients from north India in a survey was 36.7 years and study from New Delhi showed majority of patients were younger[83,84].

Majority of the patients in our study were male (67%). Data from prospective nationwide study from India showed male prepordance[19]. Study by Balakrishnan[80] et al showed male to female ratio of 2:7:1. A study from Delhi and Lucknow showed majority of their patients with tropical pancreatitis were males.

In our study mean duration of symptoms at the time of presentation was 31 months. The study from Delhi reported mean duration of 48 months while Shallu midha et al showed in their study mean duration was 27 months[85,86].

Pain was the common presentation in our study which was similar to other studies by Layer et al[81]. Balakrishnan et al[80]. Shallu midha etal[86] reported 97 % presented with pain which was similar to our data.

Diabetes was reported in 33% of patients in our study which is diferrent from study conducted by Geeverghese[87] and Tandon et al who in their study showed upto 90% of patients having diabetes. The study by Balakrishnan[80] et al showed higher incidents of diabetes upto 70%. The study from lucknow reported diabetes in 26% of patients with idiopathic chronic pancreatitis .Midha et al[86] also reported 27% patients having diabetes in chronic idiopathic pancreatitis

In our study sympotamatic steatorrhea was seen around 15%.while Midha et al[86] reported frequency of 5% steatorrhea in their study and data from New Delhi also showed around 5% of patients with steatorrohea[85].

The present study showed 78% (26) of patients with BMI > 18.5 and 22% (9) with BMI < 18.5. This is in contrast to older studies from Kerela which showed high incidence malnutrition[87,88]. Midha et al[86] and Narendranathan[89]- showed lack of association of malnutrition and Cassavsa consumption in their study. In 1988 study by Balakrishnan implicates malnutrition in pathogenesis of tropical calcific pancreatitis.

The study from lucknow and delhi showed mean BMI of 19+3 kg/m and 20.2 kg/m^2 [85].

Ultrasound and CT findings included dilated pancreatic duct, calculi, atrophy. CT was more sensitive in identifying ductal dilatation and calcification. Study from all India institute of medical science showed usefulness of ultrasonographic evaluation of calcific pancreatitis[85]. Sensitivity of identyfing ductal dilatation and calcification by ultrasound is less than CT abdomen or MRI. In our study 87.87% had parenchymal calcification 75% had ductal dilation and only 21% had ductal calculi. The study from lucknow reported 57% of their patients with tropical pancreatitis had calcification. Khuroo et al[90] reported 96 % of patients with tropical calcific pancreatis had pancreatic ductal calculi . All the 12 patients with SPINK mutation had 100% (12) parenchymal calcification and 75 % (9) atrophy of pancreas on CT.

The aetiology of idiopathic pancreatitis is not well known. Recent studies have implicated SPINK 1 and CFTR gene in idiopathic chronic pancreatitis. Study by Bhatia et al[56], Sundaresan et al[91] have shown strong association of tropical calcific pancreatitis with SPINK 1 Our study showed 36.36% of patients with SPINK mutation .The study of Bhatia et al showed SPINK 1 mutation was found in 40% of

patients with tropical idiopathic chronic pancreatitis in India. In a study from Bangladesh Schneider et al[92] showed there was difference in SPINK1 mutation between patients with tropical calcific pancreatitis having diabetes and - without. An Italian study by Macarena Gomez - showed association of SPINK 1 and CFTR gene mutation in idiopathic pancreatitis. In addition to SPINK1, CFTR gene mutations have been found in patients with CP than controls. The data from AIIMS showed 42% of patients had SPINK mutation and 9% CFTR mutation in patients with idiopathic chronic pancreatitis[61]. Studies from south India showed SPINK gene mutations were common in patients with idiopathic chronic pancreatitis .The important studies from Chen et al[93], Witt et al[13] showed significantly higher frequency of n34s mutation in spink gene in patients with idiopathic chronic pancreatitis

Thus genetic mutations seen to play an important role in the pathogeness of idiopathic chronic pancreatitis. Recent study have shown role of chymotrypsin C gene mutation in idiopathic chronic pancreatitis which lands support to the genetic theory of aeitopathogenesis of idiopathic chronic pancreatitis. The present study shows phenotypic and genetic similarities between idiopathic CP in India and in other countries.

Our study reveals that SPINK1 mutation is strongly associated with more number of acute episodes in idiopathic recurrent acute pancreatitis and parenchymal calcification in chronic pancreatitis. We need to carry out this study in more number of patients and replicate results for confirmation. Also it may be useful to do functional studies of SPINK1 mutation in cell cultures to understand the pathophysiology of disease status more completely.

SUMMARY

- The prospective study was done to evaluate clinical profile and SPINK1 genetic mutation in idiopathic recurrent acute and chronic pancreatitis.
- Total of 50 patients out of whom 17 were recurrent acute pancreatitis and 33 idiopathic chronic pancreatitis.
- Most of the patients in both the group were below 30 yrs.
- Male preponderance was seen in both groups.
- Majority of patients had BMI > 18.5 kg/m^2 in both groups.
- In idiopathic recurrent acute pancreatitis 41.17% of patients were SPINK1 mutation positive.
- 36.36% of patients with idiopathic CP were positive for SPINK1 mutation
- Patients with SPINK positive had more number of pain episodes compared to wild type in RAP groups.
- In idiopathic chronic pancreatitis pain was predominant symptom.
- In idiopathic chronic pancreatitis diabetes was seen only in 33.33 %.
- In idiopathic chronic pancreatitis all patients with SPINK positive mutation had showed parenchymal calcification by CT scan.

CONCLUSION

- This is one of the few studies in South Indian population done to assess SPINK 1 mutation and clinical correlates in idiopathic recurrent acute pancreatitis and idiopathic chronic pancreatitis.
- The prevalence of SPINK1 mutation in idiopathic RAP and CP were found to be 41.17% and 36.36% respectively.
- SPINK1 mutation patients in idiopathic RAP group had more number of acute pain episodes
- SPINK1 mutation patients in idiopathic chronic pancreatitis showed 100% parenchymal calcification by CT.
- Clinical profile of idiopathic chronic pancreatitis is different from what has been reported previously.
- Genetic testing and screening may be proposed to have role in diagnosis, predection of clinical features and severity in future.
- More replicative studies need to be done to substantiate the results.

BIBLIOGRAPHY

1. Steer ML, waxman I, Freedman S. Chronic pancreatitis. N Engl J Med 1995;332:1482 – 90.
2. Somogyi L, Martin et al. Recurrent Acute Pancreatitis: An algorithmic approach to identification and elimination of inciting factors. Gastroenterology 2001;120:708-717.
3. Whitcomb DC. Hereditary pancreatitis: A model for inflammatory disease of the pancreas. Best practice and Research Clinical Gastroenterology 2002;16:347-363.
4. Sobezynska Tomaszewska et al. Analysis of CFTR, SPINK1, PRSS1 and AAT mutations in children with acute or chronic pancreatitis. J Pediatr Gastroenterol Nur. 2006;43 (3): 299-306.
5. Whitcomb DC, Gorry MC, Preston RA et al. Hereditary Pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet. 1996;14: 141-145.
6. Funakoshi A, Miyasaka K, Jimi A et al. Protective effect of human pancreatic secretory tyrpsin inhibitors on cerulean-induced acute pancreatitis in rats. Digestion 1992;52: 145-151.
7. Eija Tukiainen, Marja-Leena K, Esko Kemppainan et al. Pancreatic secretory tyrpsin inhibitor gene mutations in patients with acute pancreatitis. Pancreas 2005; 30: 239-242.
8. Whitcomb DC. Going MAD; development of a Matrix Academic Division “to facilitate translating research to personalized medicine . Academic Medicine .J.Assoc Am Med Coll 2011;86 1353-9
9. Sarles, H. Pancreatitis symposium. Basel, SK, Marseille 1963. Revised classification of pancreatitis – Marseilles. Dig Dis Sci 1985; 30-573.
10. Mergener K, Baillie J. Chronic Pancreatitis. Lancet 1997;340: 1379-1385
11. GooryMC, Gabbaizedeh D, Furey W, Gates LK Jr, Preston RA, Aston CE, Zhang Y, Ulrich C, Enrich GD, Whitcom DC. Mutations in the cationic trypsinogen gene are

associated with recurrent acute and chronic pancreatitis. Gastroenterology 1997; 113:1063-1068

12. Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ultrich CD, Martin SP, Gates Jr LK, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Enrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996;14:141-145.
13. Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, Landt O, Becker Mutations in the gene encoding the serine protease inhibitor, Kazal tyope 1 are associated with chronic pancreatitis. Nat Genet 2000;25:213-216
14. Wang FX, Gao YJ et al. Analysis of clinical features of recurrent acute pancreatitis in Chinna. J Gastroenterol. 2006;41: 681-5
15. Gullo L et al. An update on recurrent acute pancreatitis: data from five European coutries. AM J Gastroenterol 2002; 97: 1959-62.
16. Tandon RK, Sato N, Garg PK. Chronic pancreatitis: Asia – Pacific consensus report. J Gastroenterol Hepatol 2002;5:479 – 83.
17. Tandon RK, Garg PK. Tropical pancreatitis Dig Dis 2004; 22:258-66.
18. Balaji LN, Tandon RK, Tandon BN, Banks A. Prevalence and clinical features of chronic pancreatitis in southern India. Int J Pancreatol 1994;15:29-34.
19. Balakrishnan V, Unnikrishnan AG, Thomas V, et al. Chronic pancreatitis: a prospective nationwide study of 1,086 subjects from Inida. JOP 2008;9:593-600.
20. Riela A, Zinsmeister AR. Etiology, incidence and survival of acute pancreatitis in Olmsted country, Minnesota. Gastroenterology 1991; 100: A296.
21. Moreau JA; Zinsmeister AR Melton LJ 3d. Gall stone pancreatitis and the effect of cholecystectomy: a population-based cohort study; Mayo clin Proc 1988;63 (5): 466-73.

22. Lerch MM: Saluja AK; Steer ML. Pancreatic duct obstruction triggers acute necrotizing pancreatitis in the opossum, Gastroenterology 1993; 104(3): 853-61.
23. Levy MJ, Geenen JE. Idiopathic acute recurrent pancreatitis. AM J Gastroenterol 2001;96: 2540-55.
24. Luman W; Palmer KR; Influence of cholecytectomy on sphincter of Oddi motility; Gut 1997; 41(3): 371-4.
25. Eisen G, Schutz S, Metzler D et al. Santorinicele: new evidence for obstruction in pancreas divisum. Gastrointest Endosc. 1994; 40: 73-6. 60.
26. Runzi M; Layer P; Drug associated pancreatitis: facts and fiction, Pancreas 1996; 13 (1): 100-9.
27. Fortson MR; Freedman SN; Clinical assessment of hyperlipidemic pancreatitis. Webster PD 3rd ; Am J Gastroenterol 1995; 90 (12): 213 – 9.
28. Mithofer K; Warshaw AL, Acute hypercalcemia causes acute pancreatitis and ectopic trypsinogen activation in the rat. Gastroenterology 1995; 109 (1): 239-46.
29. Schmid SW; Fries H; Malfertheiner P; Buchler MW; The role of infection in acute pancreatitis. Gut 1999; 45(2): 311-6.
30. Parenti DM; Steinberg W; Kang P; Infectious causes of acute pancreatitis Pancreas 1996; 13 (4): 356-71.
31. Sarles H. Proposal adopted unanimously by the participants of the Symposium, Marseille 1963. Bibl Gastroenterol 1965;7:7-8.
32. Singer MW, Gyr K, Sarles H. Revised classification of pancreatitis: report of the second International Symposium on the Classification of Pancreatitis in Marseille, Frace, March 28-30, 1984. Gastroenterology 1985;89:683-5.
33. Sarles H, Adler G, Dani R, et al. The pancreatitis classification of Marseille-Rome 1988. Scand J Gastroenterol 1989;24:641-2.

34. Sarner M Cotton PB. Definitions of acute and chronic pancreatitis. Clin Gastroenterol 1984;13:865-70.
35. Homma T, Harada H, Koizumi M. Diagnostic criteria for chronic pancreatitis by the Japan Pancreas Society. Pancreas 1997;15:14-5.
36. Etemad B, Whitcomb DC. Chronic Pancreatitis: diagnosis, classification, and new genetic developments. Gastroenterology 2001;120:682-707.
37. Whitcomb DC. Early trypsinogen activation in acute pancreatitis. Gastroenterology 1999; 116: 770-3.
38. Simon P, Weiss FU, Sahin-Toth M et al. Hereditary pancreatitis caused by a novel PRSS1 mutation (Arg-122> Cys) that alters autoactivation and autofegradation of cationin trypsinogen. J Biol Chem 2001;21: 21.
39. Whitcomb DC. Value of Genetic testing in the management of pancreatitis. Gut 2004; 53: 1710-1717.
40. Atkinson AJJ, Colburn WA, DeGruttola VG, et al. Biomarkers and surrogate endpoints: prferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89 – 95.
41. Schoenberg MH, Buchler M, Peitrzyk C, et al. Lipid peroxidation and gluthathione metabolism in Chronic pancreatitis. Pancreas 1995; 10:36-43.
42. Van Gossum A, Closset P, Noel E, et al. Deficiency in antioxidant factors in patients with alcohol-related chronic pancreatitis. Dig Dis Sci 1996; 4:1225-31.
43. Liska Dj. The detoxification enzyme systems. Altern Med Rev 1998; 3:187-98.
44. Lu Y, Cederbaum Al. CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med 2008; 44:723-38.
45. Park BK,Kitteringham NR, Pirmohamed M, Tucker GT.Relevance of induction of human drug-metabolizing enzymes: pharamacological and toxicological implications. Br. J Clin Pharamacol 1996; 41:477-91.

46. Telek G, Regoly-Merei J, Kovacs GC, et al. The first histological demonstration of pancreatic oxidative stress in human acute pancreatitis. Hepatogastroenterology 2001; 48:1252-8.
47. Comfort MW, Steinberg AG. Pedigree of a family with hereditary chronic pancreatitis. Gastroenterology 1952;21: 54-63.
48. Cohn JA, Friedman KJ, Noone PG et al. Relation between mutations of the cystic fibrosis gene and idiopatjic pancreatitis. N Engl J Med 1998; 339: 653-8.
49. Sharer N, Schwarz M, Malone G et al. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med 1998;339: 645-52.
50. Whitcomb DC, Yadav D, Adam S, et al. Multicenter approach to recurrent acute and chronic pancreatitis in the United States: the North American Pancreatitis study 2 (NAPS2). Pancreatology 2008;8:520 – 31.
51. Witt H, Luck W, Hennies HC, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1, are associated with chronic pancreatitis. Nat Genet 2000;25: 213-6.
52. Sossen humer M, Aston C, Ehrlich G et al 1997 clinical characteristics of hereditary pancreatits in a large family based on high risk haplotype. Am J Gastroenterol 92;1113 - 1116.
53. Ogawa M. Pancreatic secretory trypsin inhibitor as an acute phase reactant. Clin Biochem 1988; 21:19 – 25.
54. Whitcomb DC. Genetic predispositions to acute and chronic pancreatitis. Med Clin North Am 2000;84:531 – 547.
55. Threadgold J, Greenhalf W, Ellis I, et al. The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease. Gut 2002; 50:675 – 81.

56. Pfutzer RH, Whitcomb DC. SPINK 1 mutations are associated with multiple phenotypes. Pancreatology 2001;1:457 – 60.
57. Whitcomb DC. Hereditary Pancreatitis: New insights into acute and chronic pancreatitis. Gut 1999;45: 317-22.
58. Halangk W; Lerch MM: Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. J Clin Invest 2000; 106(6): 773-81.
59. Madhurkar S et al. Association of Cathepsin B gene polymorphisms with tropical calcific pancreatitis. Gut 2006; 55: 1270-75.
60. Layer P, Yamamolo H, Kaltholf L, Claen JE, Bakken LJ, Dimagno. The Different cources of Early and late onset idiopathic and alcholic chronic Pancreatitis Gastroentrology 1994;107:1481 – 1487.
61. Midha S, Khajuria R, Shastri S, et al. Idiopathic chronic pancreatitis in India: phenotypic characterization and strong genetic susceptibility due to SPINK 1 and CFTR gene mutations. Gut 2010 (in press).
62. Pfutzer RH, Barmada MM, Brunskill AP, et al. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. Gastroenterology 2000;119:615 – 23.
63. Diabetes Mellitus. Report of a WHO study group Technical Report Series 727. Wortd Health Organization, Geneva 1985.
64. Swaroop VS; Chari ST; Clain JE; Severe acute pancreatitis. JAMA 2004 16:291 (23): 2865-8.
65. Neoptolemos JP, Davidson BR, Winder AF. Role of duodenal bile crystal analysis in the investigation of “idiopathic” pancreatitis. Br J Surg 1988;75:450-453.
66. Van Gossum A, Seferian V, Rodynek JJ. Early detection of biliary pancreatitis. Dig Dis Sci 1984;29:97-101

67. Venu RP, Geenen JE, Hogan W, et al. Idiopathic recurrent pancreatitis: an approach to diagnosis and treatment. Dig Dis Sci 1989:34:56-60.
68. Satiani B, Stone HH. Predictability of present outcome and future recurrence in acute pancreatitis. Arch Surg 1979;114: 711-716.
69. Goldberg DM, Durie PR. Biochemical tests in the diagnosis of chronic pancreatits and in the evaluation of pancreatic insufficiency Clinical Biochemistry 1993;26:253 – 75.
70. Ventrucci M, Pezzilli R, Gullo L, et al. Role of serum pancreatic enzyme assays in diagnosis of pancreatic disease. Dig Dis Sci 1989;34:39 – 45.
71. Remer E, Baker M. Imaging of chronic pancreatitis. Radiol Clin North Am 2002;25:81 – 6.
72. Luetmer P, Stephens D, Ward E. Chronic Pancreatitis: reassessment with current CT. Radiology 1989;171:353 – 7.
73. Miller F, Keppke A, Wadhwa A, et al. MRI of pancreatitis and its complications: part 2, chronic pancreatitis. Am J Roentgenol 2004;183:1645 – 52.
74. Catalano MF, Sahai A, Levy M, et al. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. Gastrointestinal Endoscopy 2009;69:1251 – 61.
75. Lieb JG, Forsmark CE. Review article: Pain and chronic pancreatitis. Aliment Pharmacol Ther 2009;29:706 – 19.
76. Isaksson G, Ihse I. Pain reduction by an oral pancreatic enzyme preparation in chronic pancreatitis. Dig Dis Sci 1983;28:97 – 102.
77. Gachago C, Draganov PV. Pain management in chronic pancreatic enzyme preparation in chronic pancreatitis. World J Gastroenterol 2008;14:3137 – 48.
78. Garg PK; Tandon RK; Madan K; Is biliary microlithiasis a significant cause of idiopathic recurrent acute pancreatitis? A long-term follow up study. Clin Gastroenterol Hepatol. 2007;5(1): 75-9.

79. Giulia Martina C, Raffella A, Simone B, Giuliana s, Luca F, Stefanao, at al connection between genetics and clinical data role of MCP – I, CFTR, SPINK in Acute Recurrent and chronic pancreatitis. Amj J Gastroenterol 2010;l05:199 – 206.
80. Balakrishnan V, Nair P, Radhakrishnan L, et al.Tropical pancreatitis- a distinct entity, or merely a type of Chronic pancreatitis? Indian J Gastroenterol 2006: 25:74-81.
81. Layer P, Yamamoto H, Kaithoff L, et al. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. Gastroenterology 1994; 107:1481-7.
82. Kandula L, Whitcomb DC, Lowe ME, Genetic issues in pediatric pancreatitis. Curr Gastroenterol Rep 2006; 8:248-53.
83. Garg PK, Tandon RK. Survey on chronic pancreatitis in the Aisa Pacific egion. J Gastroenterol Hepatol 2004;19: 998-1004.
84. Choudhuri G, Bhatia E, Sikora SS, Alexander G. Tropical pancreatitis in North India. In: Chronic pancreatitis and Pancreatic Diabetes in India Balakrishnan V, Kumar H, Sudhindran S, Unnikrishnan AG, eds (Indian Pancreatitis study Group) 2006:53-9.
85. Garg PK. Chronic Pancreatitis: The AIMS, New Delhi experience. In: Chronic Pancreatitis and Pancreatic Diabetes in India Balakrishnan V, Kumar H, Sudhindran S, Unikrishnan AG, eds (Indian Pancreatitis Study Group) 2006: 61-76.
86. Midha S, Singh N, Sachdev V, et al. Cause and effect relationship of malnutrition with idiopathic chronic pancreatitis: prospectyive case control study. J Gastroenterol Hepatol 2008;23:1378-83.
87. Geeverghese PH. Pancreatic diabetes: a clinicopathological study of growth onset diabetes with pancreatic calculi. Mumbai, India: Popular Prakashan; 1968.
88. Balakrishnan V, Sauniere, Hariharan M, et al. Diet, pancreatic function and chronic pancreatitis in South India and France. Pancreas 1988; 3:30-5.

89. Narendranathn M, Cheriyan A. Lack of association between cassava consumption and tropical pancreatitis syndrome. J Gastroenterol Hepatol 1994;9:282 – 5.
90. Khuroo NS, Khuroo MS, Khuroo MS. anomalours pancreaticobiliary ductal union in tropical calcific pancreatitis. JOP.J Pancreas (Online) 2010;11:18 – 24.
91. Sundaresan S, Chacko A, Dutta AK, et al. Divergent roles of SPINK 1 and PRSS2 variants in tropical calcific pancreatitis. Pancreatology 20099;9:145 – 9.
92. Schnerder A, suman A, Rossi L, et al. SPINK1/PSTI mutations are associated with tropical pancreatitis and type II diabetes mellitus in Bangladesh. Gastroenterology 2002;123:1026 – 30.
93. Chen JM, Mercier B, Audrezet MP, Ferec C. Mutational analysis of the human pancreatic secretory trypsin inhibitor (PSTI) gene in hereditary and sporadic chronic pancreatitis. J Med Genet 2000;37:67 – 9.

PROFORMA

1. Name :

2. Age :

3. Sex :

4. Date of Admission :

5. Presenting Complaint :

6. Past History :

7. Examination : Pulse BP Respiratory System

CVS Abdomen

8. Investigations : CBC Creatinine

FBS Calcium

Lipid Profile Thyroid profile

PTH ANA IG4 levels

9. Serology Test

10. Bile for Micorlithiasis

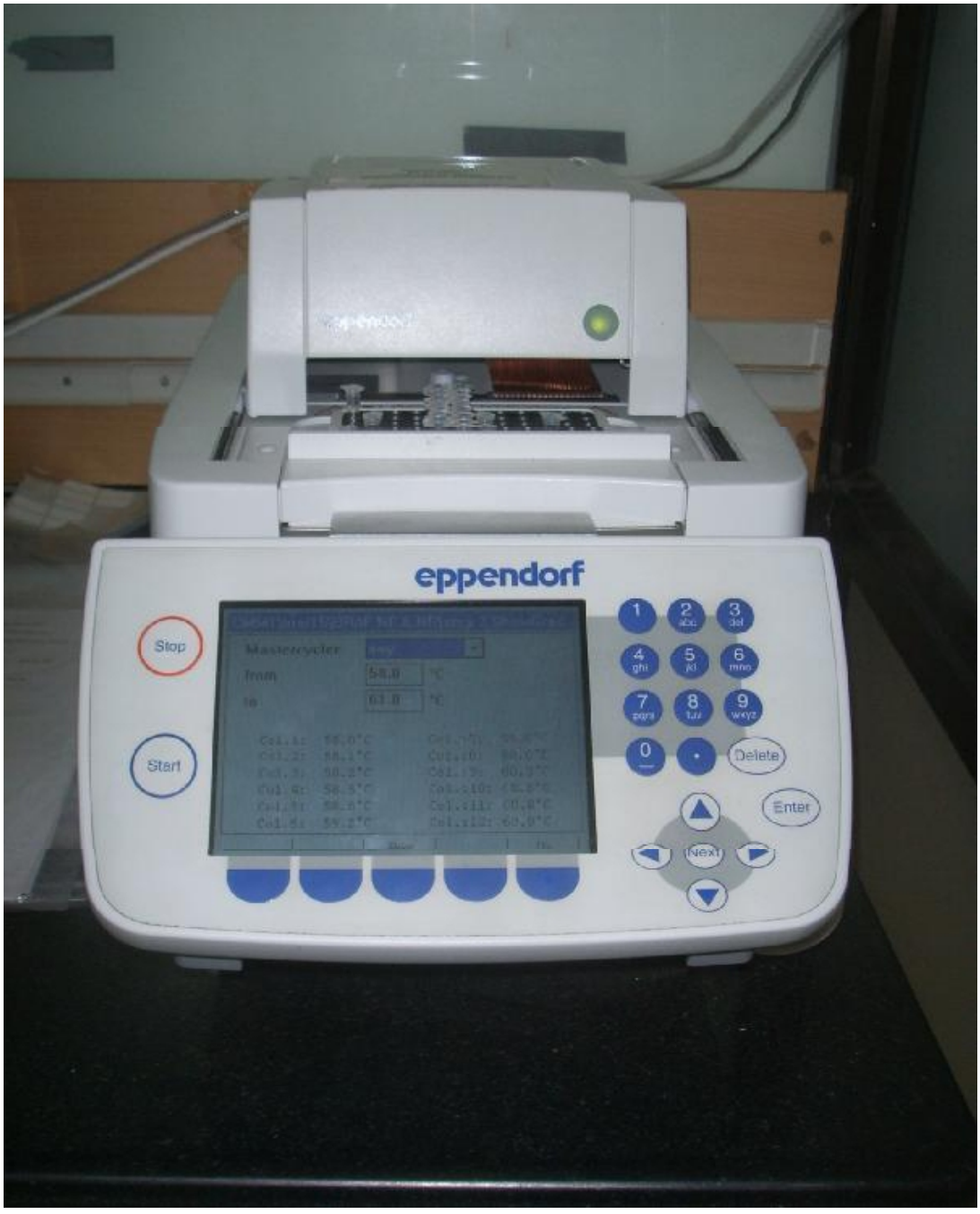
11. Ultra Sound

12. CT Abdomen

13. EUS/ MRCP

14. Genetic Analysis

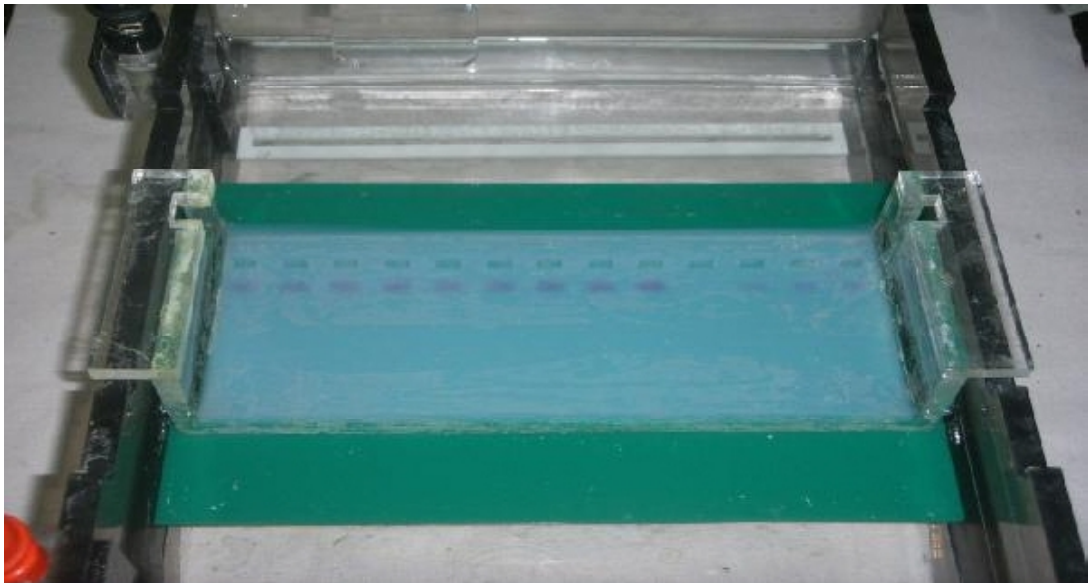
COLOUR PLATE



Picture showing PCR amplification of DNA

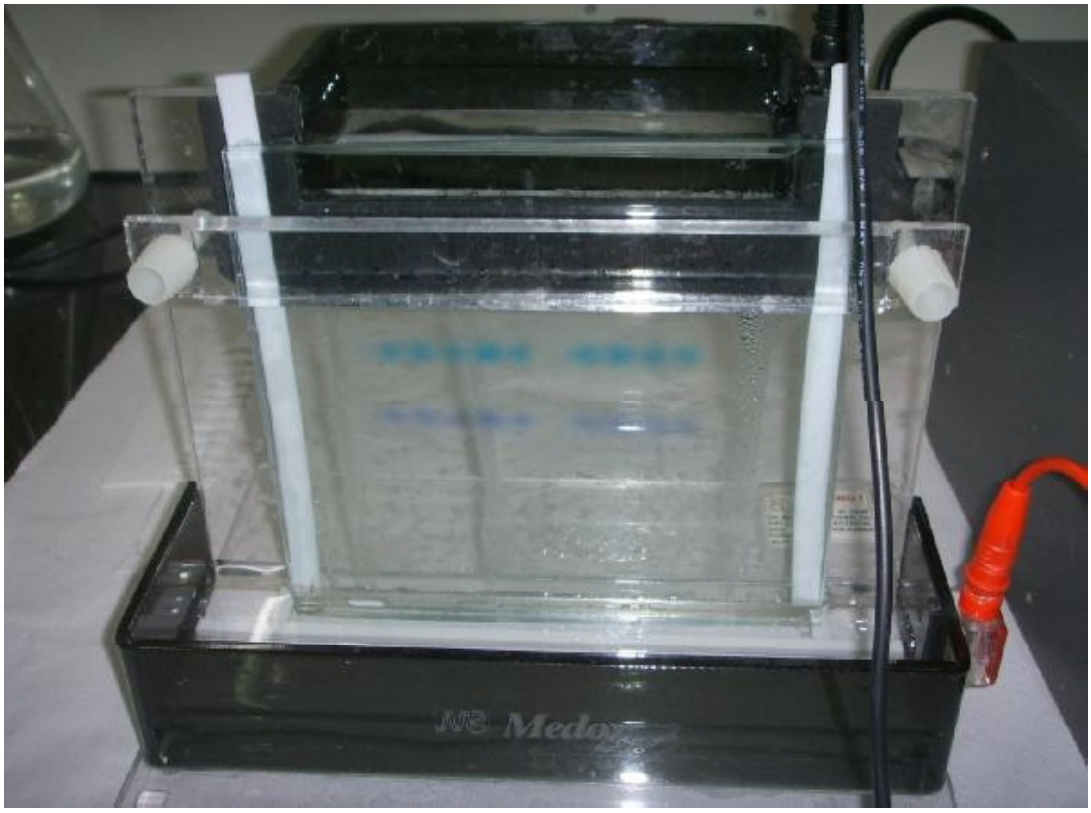
COLOUR PLATE

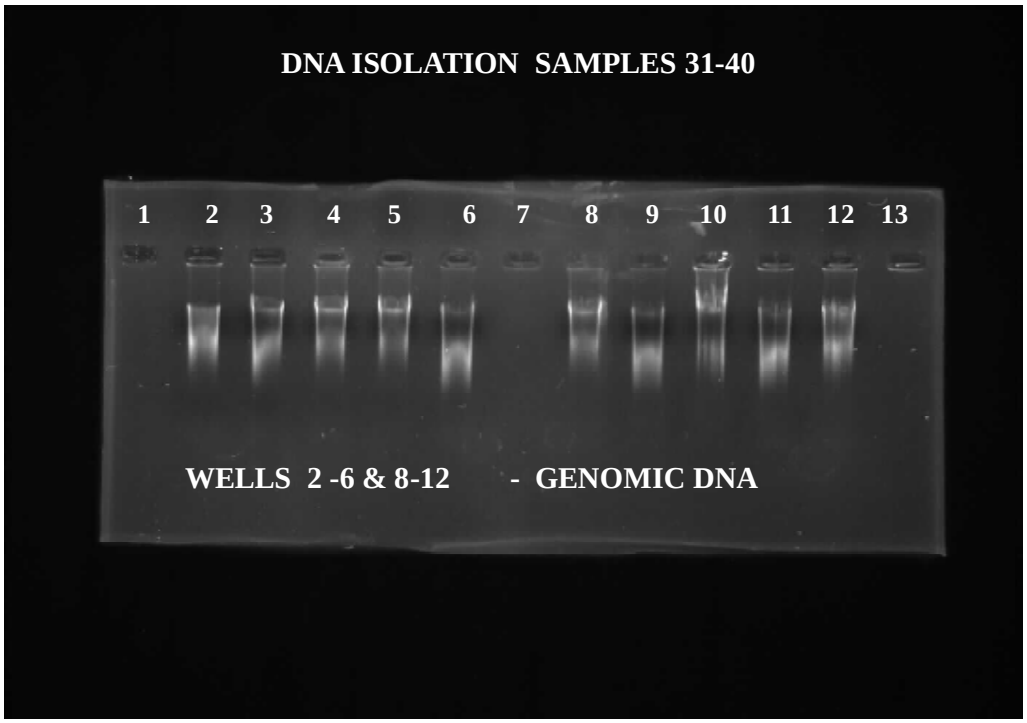
Picture showing 2% agarose gel electrophoresis of PCR Product

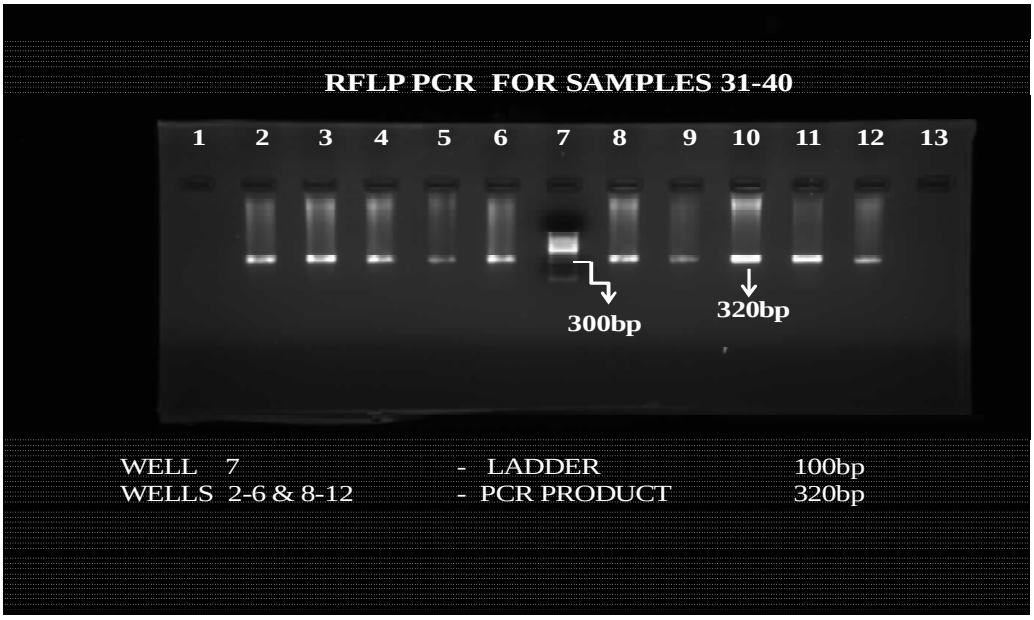


COLOUR PLATE

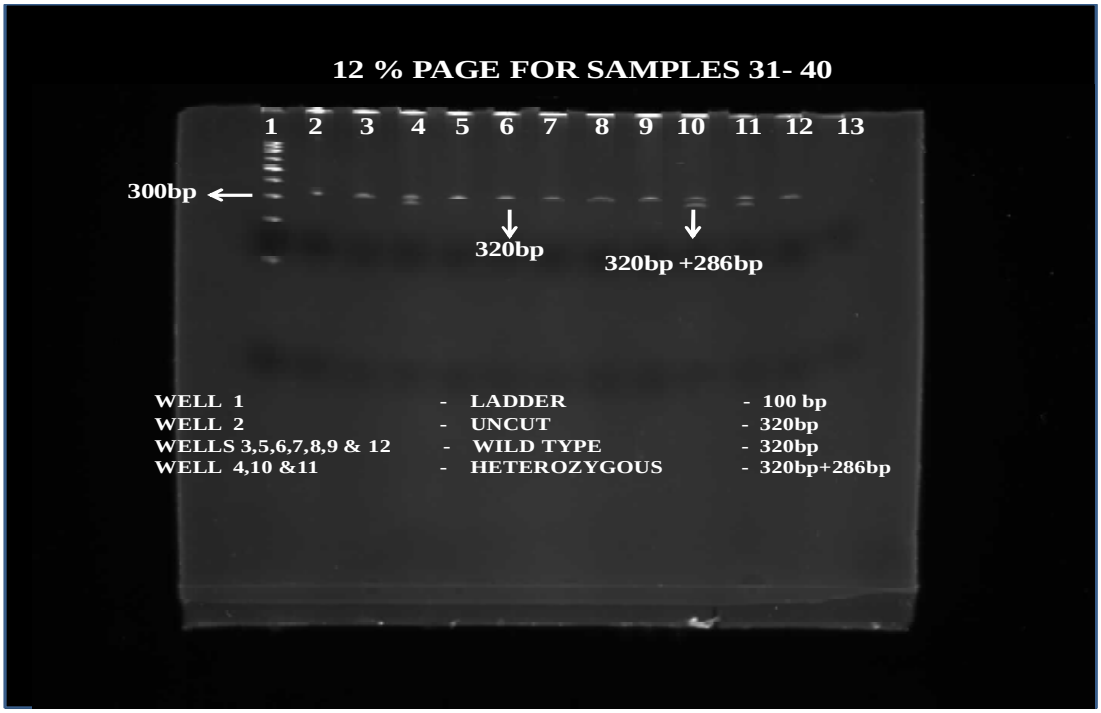
Picture showing 12% Polyacrylamide gel electrophoresis of digested RFLP




DNA ISOLATION SAMPLES 31-40
1 2 3 4 5 6 7 8 9 10 11 12 13
WELLS 2 -6 & 8-12 - GENOMIC DNA

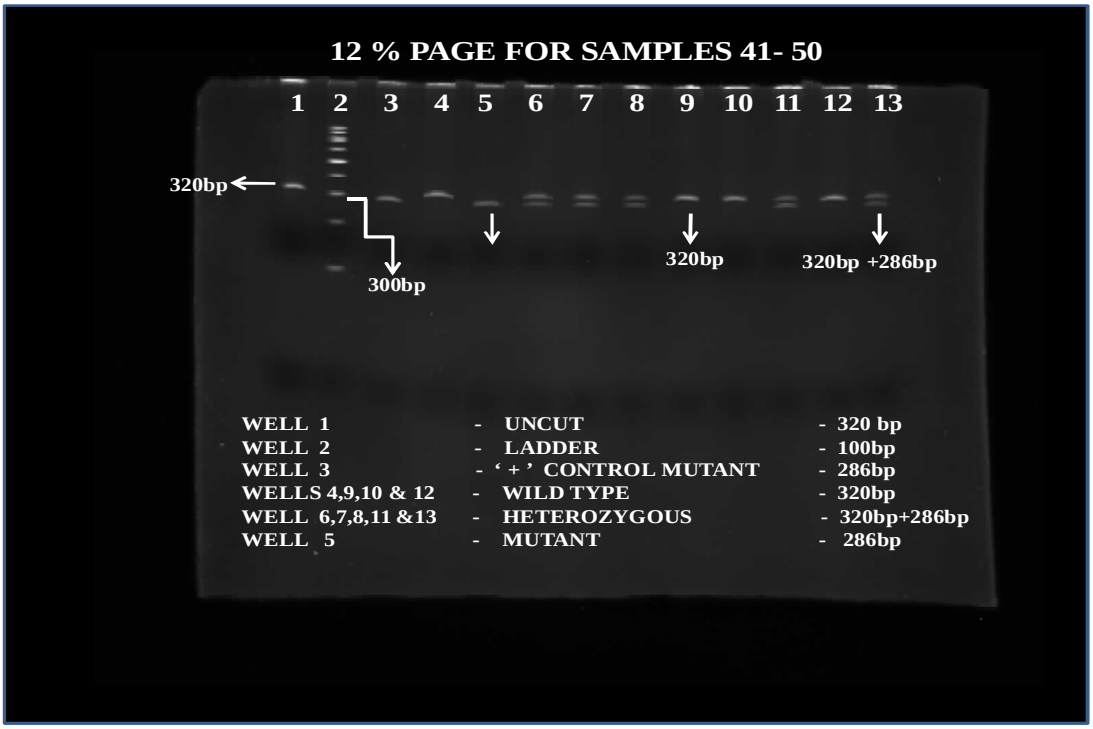

RFLP PCR FOR SAMPLES 31-40
1 2 3 4 5 6 7 8 9 10 11 12 13
300bp
320bp
WELL 7 - LADDER 100bp
WELLS 2-6 & 8-12 - PCR PRODUCT 320bp

12 % PAGE FOR SAMPLES 31- 40



WELL 1	- LADDER	- 100 bp
WELL 2	- UNCUT	- 320bp
WELLS 3,5,6,7,8,9 & 12	- WILD TYPE	- 320bp
WELL 4,10 &11	- HETEROZYGOUS	- 320bp+286bp

12 % PAGE FOR SAMPLES 41- 50



WELL 1	- UNCUT	- 320 bp
WELL 2	- LADDER	- 100bp
WELL 3	- ‘ + ’ CONTROL MUTANT	- 286bp
WELLS 4,9,10 & 12	- WILD TYPE	- 320bp
WELL 6,7,8,11 &13	- HETEROZYGOUS	- 320bp+286bp
WELL 5	- MUTANT	- 286bp

ABSTRACT

Recurrent acute pancreatitis and chronic pancreatitis are labeled as idiopathic when no identifiable factors are found. The identifications of genetic mutations associated with pancreatitis have provided opportunities for identifying patients at risk for idiopathic pancreatitis.

Aim:

To study of clinical profile and prevalence of SPINK 1 mutation in idiopathic recurrent acute and chronic pancreatitis.

Design:

Prospective observational study of patients with idiopathic recurrent and chronic pancreatitis in a tertiary care hospital from November 2010 to 31st October 2011.

Results:

Fifty patients were included out which 17 patients were idiopathic recurrent acute pancreatitis and 33 were chronic. Out of 17 patients with RAP mean age was 22.29 ± 9.7 years, duration of illness was 28.23 ± 10.34 months, 82% were male, 94% had BMI > 18.5 kg/ m^2 41.17% had SPINK1 mutation. Out of 33 patients with chronic pancreatitis mean age was 31.75 ± 13.07 year, duration of illness was 31.33 ± 19.89 months, mean fasting sugar was 112.57 mg/dl, 67% were male, 93.94% had pain 87.8% had ductal dilatation on CT, 36.36% were SPINK 1 positive.

Conclusion:

SPINK1 mutation patients have more frequent episodes of pancreatitis and parenchymal calcification on CT. The clinical profile of idiopathic chronic pancreatitis is different from what has been reported in the past.

Key words:

Idiopathic Recurrent Acute Pancreatitis; Chronic Pancreatitis SPINK1 mutation