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Original Article

Internal Medicine/ Gastroenterology Section

Spink1 Mutation in Idiopathic Recurrent Acute Pancreatitis- A Pilot Study

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ABSTRACT

Introduction: Recurrent Acute Pancreatitis (RAP) and Chronic Pancreatitis (CP) are labeled as idiopathic when no identifiable factors are found. The identification of genetic mutations associated with pancreatitis have provided opportunities for identifying patients at risk for idiopathic pancreatitis.

Aim: The aim of the present study was to study the clinical profile and prevalence of *SPINK1* mutation in idiopathic RAP.

Materials and Methods: The present study was a prospective observational study of idiopathic RAP patients at a tertiary care hospital. DNA was isolated from blood obtained from patients and genotyping of *SPINK1* mutation was studied.

Results: A total of 17 patients with idiopathic RAP were included. Their mean age was 22.29±9.7 years, 14 (82%) were male, 7 (41.7%) had *SPINK1* mutation. Patients with *SPINK1* mutations had more frequent acute episodes of pancreatitis.

Conclusion: *SPINK1* mutation patients have more frequent acute episodes of pancreatitis and nearly 7 (41.7%) had *SPINK1* mutations in the so called idiopathic RAP. Genetic testing in idiopathic pancreatitis might have role in future. Multicenter studies are further required to confirm the role of genetic testing in RAP.

Keywords: Gastroenterology, Gene mutation, Prevalence

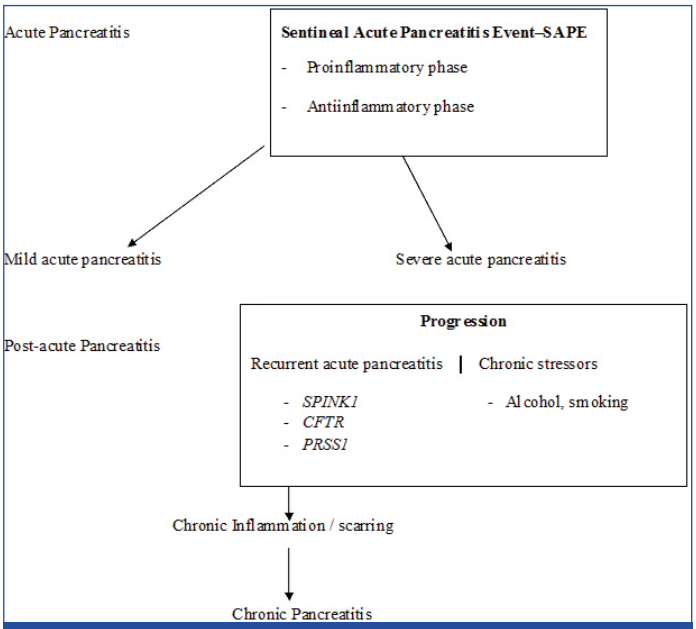
INTRODUCTION

Pancreatitis is the occurrence of inflammation in an otherwise normal gland and is commonly seen when patients present with severe abdominal pain. It is diagnosed by appropriate imaging and with the help of laboratory markers viz., Pancreatic amylase and lipase [1]. Acute pancreatitis can contribute to the development of RAP if aetiology remains unknown. The RAP is defined as two or more attacks of acute pancreatitis with symptom free intervals. Evaluation and appropriate treatment is vitalin RAP since >50% of patients experience recurrent episodes that contributes to the development of CP [2,3]. In clinical practice routine evaluation often fails to detect the cause of pancreatitis in 10-30% of the patients, and these patients are referred as idiopathic chronic or RAP [4]. A very few studies have been conducted on idiopathic RAP. The RAP can be due to biliary disease, alcohol, trauma, hypercalcemia, hyperlipidemia, or anatomical variations [3,4].

Mutations in *Cationic Trypsinogen* gene (*PRSS1*), *SPINK1* gene, *Cystic Fibrosis Transmembrane Conductance Regular (CFTR)* gene and *Cathepsin B* gene have been studied in RAP and CP [5-8]. 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 blocking the active site, 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 [7,8]. Recently, the role of *SPINK1* mutation in CP has emerged. The most frequent mutation in *SPINK1* gene exon 3 results in asparagine to serine amino acid change (N34S) which leads to decreased trypsin inhibitory capacity. *SPINK1* (N34S), mutations are relatively common in 2% of general population, shown to be associated with pancreatitis [9]. *SPINK1* mutation is hypothesised to be a susceptibility factor or a disease modifier in recurrent pancreatitis [10].

Genetic mutations may be the cause of pancreatitis in patients where aetiology remains undiagnosed [Table/Fig-1] [11,12]. 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 [13]. A small pilot systematic study on clinical profile and prevalence of *SPINK1* mutation was studied in patients with idiopathic RAP.

In the present study we aim to study the demographic and clinical profile of idiopathic RAP and to assess the prevalence of genetic mutation (*SPINK1*) in idiopathic RAP.



[Table/Fig-1]: Progression from pre-acute to chronic pancreatitis.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Human Ethics Committee (IHEC) prior to the start of the study. Written informed consent was obtained from patients prior to study entry. A total of 17 patients who met the inclusion criteria of idiopathic RAP were enrolled. Since the disease is rare, we wanted to include all possible cases, hence no sample size was separately determined.

Inclusion Criteria

The inclusion criteria was selected on the basis of prior studies [9,11,12]. Two documented episodes of typical pancreatic type of abdominal pain, amylase or lipase greater than three times the upper limit of normal, features of acute pancreatitis on imaging studies (ultrasound/CT abdomen), no identifiable cause or risk factors.

Exclusion Criteria

Patients with identifiable cause and risk factors for acute and recurrent pancreatitis, malignancy, retroviral infection, psychiatric illness, hereditary and known genetic diseases.

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 work up Ig4 levels viral serology and Endoscopic Ultrasound (EUS) for microlithiasis and sludge after informed consent. The following imaging studies viz., transabdominal ultrasonography, Contrast enhanced CT abdomen, Magnetic resonance cholangiopancreatography/ EUS and *SPINK1* mutation testing were done.

Genotyping: Genomic DNA was extracted from blood using QIAamp DNA Blood Mini Kit (Qiagen, USA). Genotyping initiated with DNA extraction, followed by Polymerase Chain Reaction (PCR) to amplify *SPINK1* gene, RFLP using PstI restriction enzyme and Poly Acrylamide Gel Electrophoresis (PAGE) of the digested product [14]. The PCR reactions were performed on Eppendorf thermocycler. Primers used were forward primer-SPINKR: TTCTGTTTAATTCCATTTTTAGGCCAAATGCTGCA; reverse primer-SPINKR: GGCTTTTATCATACAAGTGACTTCT. DNA was isolated using Micro AX Blood Gravity Kit (A&A Biotechnology, Gdańsk, Poland). Quality and concentration of isolated DNA was measured by Nano Drop 2000 (Thermo Scientific, TK Biotech, Poland). Genotyping was performed using Allele-Specific PCR (ASA-PCR) and High-Resolution Melting (HRM)-PCR as described previously [15]. The genotypes were determined based on the expected product size [Table/Fig-2].

Genotype	Mutation Site	Product Size (bp)
A/A	(Asn 34 Asn)	320 bp
G/G	(Ser 34 Ser)	286 bp
A/G	(Asn 34 Ser)	320, 286 and 34

[Table/Fig-2]: Genotypes with their expected product size.
Asn: Asparagine; Ser: Serine; bp: Base pairs

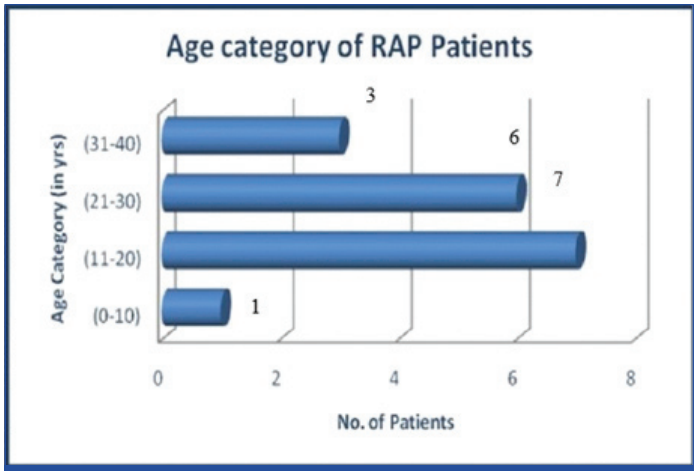
STATISTICAL ANALYSIS

Study variables were analysed by statistical package SPSS (Version 10.0. Descriptive statistics mean and standard deviation were used, number and percentage are given where appropriate.

RESULTS

A total of 17 patients were included and their mean age was 22.29±9.70 years, the youngest enrolled person was 14-year-old and the oldest was 39-year-old with 14 patients below 30 years of age [Table/Fig-3]. 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. A total of 14 (82%) patients were male and 3 (18%) were female, suggesting male preponderance. Majority of the patients had BMI >18.5 kg/m^2.

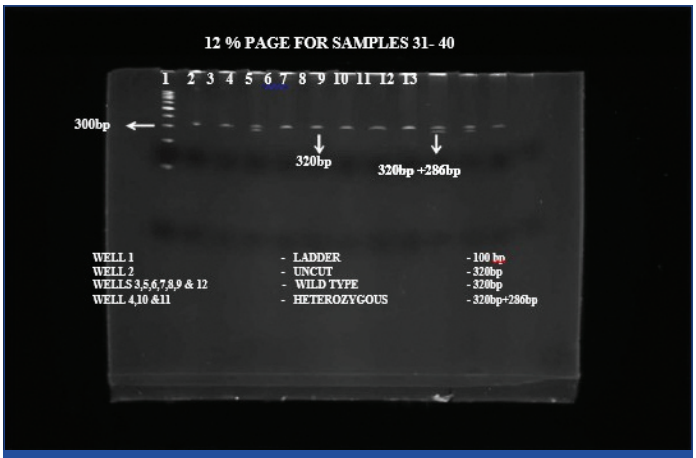
Majority of patients had three episodes of pancreatitis [Table/Fig-4]. Patients with *SPINK1* mutation positive had more number of pancreatitis episodes compared to wild type. All the *SPINK1* positive patients had four and above pancreatitis episodes. Out of 17 patients with idiopathic RAP 7 (41.17%) were positive for *SPINK1* mutation. A total of 10 (58.3%) had wild type and 7 (41.7%) had heterozygous genotype [Table/Fig-5a,b].



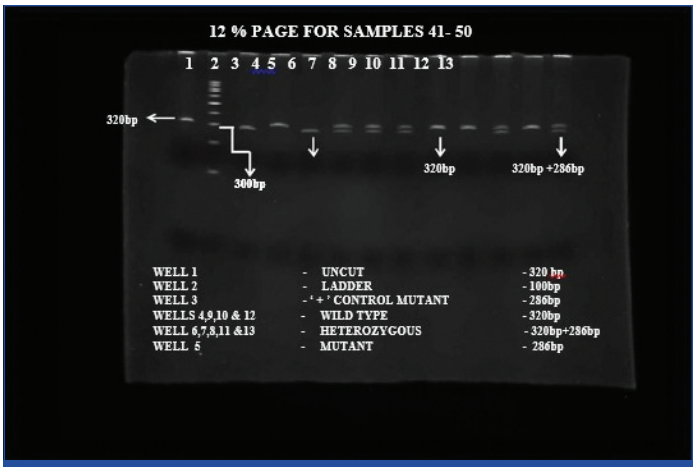
[Table/Fig-3]: Age among RAP patients at enrollment.

Pancreatitis Episodes	No. of Patients	No. of *SPINK1* positive
≤3	8	-
3-4	2	5
4-5	-	1
> 5	-	1

[Table/Fig-4]: Pancreatitis episodes among RAP with *SPINK1 mutation*.



[Table/Fig-5a]: PCR gel image depicting bands of various phenotypes and genotypes.



[Table/Fig-5b]: Heterozygous and wild *SPINK1* mutation on PCR gel image.

DISCUSSION

Acute pancreatitis can lead to recurrent episodes and cause CP. In practice many a times no cause can be made out and such cases are labeled as idiopathic pancreatitis. Many studies are focused on idiopathic CP; however, only few have been on truly idiopathic recurrent pancreatitis, so we conducted a short pilot study. The present study was a prospective analysis of 17 patients diagnosed to have idiopathic RAP. The present study was conducted on South Indian population

to assess *SPINK1* mutation and clinical profile in idiopathic RAP. Male dominance was evident as seen in two prior studies around the world [17,18]. It is currently unknown as to why this is more commonly seen in male patients. The mean age of the patients in two prior studies were 41 and 43 years, respectively [16,17]. while, the mean age of the patients in present study was 22.29 years. All patients in the present study had recurrent episodes of pancreatitis (>2) which was similar to a North Indian study [18]. The present study had 17 patients out of which 7 (41.17%) were positive for *SPINK1* mutation and all seven patients had increased episodes of pancreatitis compared to wild type (no mutation). Another study by Wkitcomb DC, shown significant cases of idiopathic RAP have genetic components like *SPINK1*, CFTR [19]. Genetic theory on pancreatitis says that carrying *SPINK1* mutation leads to increased episodes of acute pancreatitis and leads to CP later [20,21]. However, another study by Cavestro GM et al., did not find a statistically significant association with this mutation [22]. Hence there is a need to assess additional mutations which could be present in addition to *SPINK1* mutation.

Genetic mutations seem to play an important role in the pathogenesis of idiopathic CP. Recent studies have shown a role of *Chymotrypsin C* gene mutation in idiopathic CP which lands support to the genetic theory of aetiopathogenesis of idiopathic CP. The present study revealed that *SPINK1* mutation is strongly associated with more number of acute episodes in idiopathic RAP. We need to carry out the present 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. Molecular and genetic analysis will become important in future. Mutation will provide information on risk of developing pancreatitis. Mutation detection will assist in early diagnosis, mutation identified will provide rationale classification. Molecular classification will help in knowing disease progression and prognosis. Specific mutation will help in gene-environmental interaction. Mutation may help in developing new therapeutic intervention. Such studies will help patients to understand as to why some develop pancreatitis while rest don't.

LIMITATION

Being single centered with a smaller sample size limits generalisability of our findings. Limitation also arises from the selected study approach as to not include mutations other than *SPINK1*.

CONCLUSION

By prospectively analysing RAP patients in a tertiary care center, we studied *SPINK 1* mutation. *SPINK 1* mutation patients have frequent episodes of pancreatitis and two fifth of the study population had *SPINK 1* mutations. Genetic testing has an evident role in future for idiopathic RAP. The need of the hour is to have genetic mutational studies at various centers with a bigger sample size to identify new mutations.

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