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Original Research Article

Identification of Genetic Risk Factors in Chronic Pancreatitis in South Indian Population –A Pilot Study

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Abstract:

Background: Chronic pancreatitis is an irreversible process and potentially life-threatening disease, in most cases a causative factor will be alcohol (70%). In about 10-30% of the patients the etiology is undetermined, these categories are labelled as idiopathic chronic pancreatitis. Unexplained recurrent acute pancreatitis however may be associated with known genetic mutations in the cationic trypsinogen gene (PRSS1), the SPINK1 gene, or the CFTR gene. Furthermore, a better understanding of the interactions of the etiological factors with susceptibility SNPs will aid in diagnosing and treating the disease at an early stage. Based on this aim is to study the prevalence of SPINK1 and PRSS1 mutation in patients with chronic pancreatitis in south Indian population.

Methodology: This study was done as an observational cross sectional study among patients with chronic pancreatitis, recurrent abdominal pain, idiopathic chronic pancreatitis, persons with family history of pancreatitis. We did the study in 42 patients between September 2019-August 2021 at Government Rajaji hospital, Madurai. SPINK1 and PRSS1 mutation analysis was done in all patients.

Results: We did this study in 42 patients of chronic pancreatitis. In our study population abdominal pain was the main complaint in all patients. Loss of appetite (n=28) and loss of weight (n=24) is the next common feature among our study participants. In our study population, no patients had family history of pancreatitis. In our study 23 out of 42 patients had recurrence of episodes. 4 among 42 patients in our study had levels in CA 19.9 positive. No significant relation was there with the SNP. In our study heterozygous PRSS1 – positive for mutation was present in all patients in our study (100%). SPINK1 mutation was positive in 18 patients (43%) in our study.

Conclusion: Mutation of PRSS1 and *SPINK1* may predispose patients to acute pancreatitis, especially in those abusing alcohol, and may promote a more severe course of the disease. Having an idea which polymorphisms confer susceptibility to acute pancreatitis, and which polymorphisms modify the immunological response to acute and recurrent acute pancreatitis that lead to chronic pancreatitis will also be important in determining the timing of genetic testing and subsequent intervention. Genetic testing in future will help in avoiding progress to Chronic or recurrence in pancreatitis patient by taking rational preventative measures.

Keywords: Pancreatitis, PRSS1, SPINK1, Mutation.

Introduction

Chronic pancreatitis is a potentially life threatening disease, in most cases a causative factor will be alcohol (70%). In about 10-30% of the patients the etiology is undetermined, these categories are labelled as idiopathic chronic pancreatitis. There has been considerable recent interest in the few patients who develop pancreatitis on a hereditary basis. It is generally believed that spontaneous trypsinogen activation normally occurs to a slight degree within the pancreas but that, in normal, the pancreas is protected from injury by the presence of trypsin inhibitors. [1] In Hereditary pancreatitis with genetic mutations, protective process fails either because a trypsin that is resistant to inhibition is synthesized or the trypsin inhibitors themselves are defective. Hence intrapancreatic activation of trypsin and other digestive enzymes and repeated episodes of pancreatitis happens. In hereditary pancreatitis, attacks begin at a young age and lead to chronic changes, including fibrosis,

calcifications, and loss of both exocrine and endocrine functions. The incidence of pancreatic cancer is also markedly increased in patients with hereditary pancreatitis. Hereditary pancreatitis is an autosomal dominant disease with incomplete penetrance. [2]

The genetic studies in chronic pancreatitis can be used to identify the susceptible persons, the better understanding of genetic studies will aid in diagnosing and treating the patients at an early stage & strategies should be discussed to prevent future episodes of acute on chronic pancreatitis by avoiding concomitant risk factors like alcohol, metabolic disturbance and drugs. As these mutations increases the risk of pancreatic cancer these patients should be counselled for abstinence from alcohol, smoking and to identify at risk relatives.

In most cases, acute pancreatitis is caused by gall stones or alcohol and no genetic testing is indicated. Unexplained recurrent acute pancreatitis however may be associated with known genetic mutations in the cationic trypsinogen gene (PRSS1), the SPINK1 gene, or the CFTR gene. Other genes are also likely important but they are yet to be discovered and understood within the context of pancreatic disease.[3,4]

Most cases of hereditary pancreatitis are associated with mutations in the cationic trypsinogen gene (PRSS1) [5,6]. Although nearly 20 pancreatitis associated mutations in the PRSS1 gene have been described, the R122H and N29I mutations (also numbered as R117H and N21I using the chymotrypsinogen amino acid numbering system) represent the vast majority of cases. These are gain of function mutations that likely interfere with autolysis and/or cause premature trypsinogen activation. In families with PRSS1 R122H or N29I mutations, between 60% and 80% of individuals who inherit the mutation usually develop pancreatitis.[7,8] In addition, approximately half of individuals with acute pancreatitis will develop chronic pancreatitis, and up to 40% of individuals with chronic pancreatitis will develop pancreatic cancer. In most cases, a patient with a gain of function PRSS1 gene will identify at least one other family member with recurrent acute or chronic pancreatitis.

PRSS1 genetic testing is recommended in symptomatic patients with any of the following: (1) recurrent (two or more separate documented episodes of typical pain with hyper-amylasaemia) attacks of acute pancreatitis for which there is no explanation (for example, anatomical anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gall stones, alcohol, drugs, hyperlipidaemia, etc.), (2) unexplained (idiopathic) chronic pancreatitis, (3) a family history of pancreatitis in a first degree (parent, sib, child) or second degree (aunt, uncle, grandparent) relative, (4) an unexplained episode of documented pancreatitis occurring in a child that has required hospitalisation, and where there is significant concern that hereditary pancreatitis should be excluded, or (5) as part of an approved research protocol. [9]

Furthermore, heterozygous SPINK1 or CFTR mutations appear more commonly in idiopathic pancreatitis than homozygous or compound heterozygous mutations in these genes, as predicted for autosomal recessive diseases. Finally, heterozygous SPINK1 and CFTR gene mutations are often enriched in familial pancreatitis families that may not follow classic Mendelian inheritance patterns. This suggests that these mutations are components of complex genetic traits. Genetic testing will become important as the critical factors and interactions are identified and interpretation and prediction become possible.

Although the SPINK1 N34S allele is associated with multiple types of acute and chronic pancreatitis, the association is weak, with, 1% of mutation carriers developing pancreatitis sometime during their lifetime. [10] Furthermore, the severity of pancreatitis appears to be similar between subjects with heterozygous, homozygous, or compound heterozygous genotypes that include the N34S allele suggesting that the genetics is complex. Therefore, we hypothesised that the SPINK1 N34S allele was acting as either a modifier gene or a susceptibility factor for a polygenic complex trait. [11,12] In addition, other rare SPINK1 polymorphisms have been identified that are associated with a higher risk of pancreatitis than the N34S genotype

Furthermore, a better understanding of the interactions of the etiological factors with susceptibility SNPs will aid in diagnosing and treating the disease at an early stage. Based on this aim of the study is to evaluate the prevalence of SPINK1 and PRSS1 mutation in patients with chronic pancreatitis in south Indian population.

Material and Methodology

This study was done as an observational cross sectional study among patients with chronic pancreatitis, recurrent abdominal pain, and idiopathic chronic pancreatitis. We did the study in 42 patients between September 2019-August 2021 at Govt Rajaji hospital, Madurai. All Patients aged more than 14 years diagnosed with chronic pancreatitis and who have given consent are included in the study. While patients with age less than 14 years and not given consent are excluded from the study. The study was approved by the Institutional Ethics Committee of Madurai Medical College, Madurai and performed as per the

standards laid down by the Declaration of Helsinki for medical research involving human subjects.

About 3 ml of vein blood sample was collected from 42 patients with chronic pancreatitis in the Dept. of Surgical Gastroenterology, Government Rajaji Hospital, Madurai. The samples was transported to Multidisciplinary research Unit, Subsequently DNA was isolated from blood samples by using the Qiagen DNA isolation kit as per the manufacturer's instructions. The quantity and quality of the DNA was determined using Nano Drop (Thermo, USA).

Genetic analysis

DNA was subjected to polymerase chain reaction (PCR) amplification. For PRSS1 gene (PRSS1 gene, GenBank TM accession no. U66061) mutation detection of exon 3 the cationic trypsinogen was amplified using specific primers. After, PCR products are subjected to BstUI restriction endonuclease. PCR-amplified restricted products were electrophoretically separated on agarose gel electrophoresis using a 2% agarose stained with ethidium bromide. A restriction site analysis was undertaken to identify restriction enzymes that would permit to establish a rapid screening method that detects the novel mutation. For SPINK1 mutation detection DNA samples from the patients were analyzed for a common mutation of SPINK1 was N34S mutation (GenBank, NM-003122).) of exon 3 was detected by PCR-restriction fragment length polymorphism (RFLP) using specific primer sequences.

The primers were designed to introduce a PstI endonuclease restriction site in sequences containing the N34S mutation. PCR were performed in an automated thermal cycler Biorad, USA). The PCR products were then digested with restriction endonulcease PstI. Undigested amplification products were 320 bp long. After digestion with PstI, a product of 286 bp was obtained from mutant sequences. Heterozygote samples produced products of both 320 and 286 bp. PCR-amplified products were electrophoretically separated on agarose gel electrophoresis using a 2% agarose stained with ethidium bromide.

Results

We did this study in 42 patients of chronic pancreatitis, among this 10 patients were less than 30 years, and 12 patients were in 31-40 years range, 16 patients in 41-50 year range and rest 4 patients above 50 years of age. In our study 29 patients were male and 13 patients were female.

In our study population abdominal pain was the main complaint in all patients. Pain was most common in epigastric region; mostly it was dull aching in nature. Most of patients had radiation to back and pain responded to analgesics. In our study population of 42 patients 36 patients had gone to hospital in past one year at least once for getting treated for pain.

Loss of appetite (n=28) and loss of weight (n=24) is the next common feature among our study participants. Anorexia, vomiting and steatorrhea were present in few cases in our study and jaundice and melena was also seen.

Diabetes (n=14) was most common comorbidity associated in patients included in our study group. Two patients had TB. 24 patients were alcoholic and 23 patients were smoker in our study group. 2 patients were HbsAg positive.

In our study population, no patients had family history of pancreatitis. Surgery required in 3 patients in our study. We analyzed recurrence and in our study 23 out of 42 patients had recurrence of episodes. Also we analysed CA 19.9 levels.

Values above 37 were taken as positive and 4 among 42 patients in our study had levels in positive range. We next did single nucleotide polymorphism of two genes in our study –PRSS1 and SPINK1. In our study heterozygous PRSS1 – positive for mutation was present in all patients in our study (100%). SPINK1 mutation was positive in 18 patients (43%) in our study.

Table 1: Distribution of mutation

GENE	No of mutation	Percentage
PRSS1	42/42	100%
SPINK1	18/42	43%

We further analyzed this SNP results with various related factors. To start with Age distribution, As PRSS1 mutation was present in all cases, the above normal distribution was present in same way, while among 18 SPINK1 mutation cases most of cases were in 41-50 years age group. There was no significant relation between age and SPINK1 mutation with p value of 0.133. Moving on to gender distribution, among 18 patients having SPINK1 mutation 11 were male and rest 7 was female and this was not statistically significant with a p value of 0.335. In our study population of 42 patients and only 4 had increased CA 19.9 levels and among which 3 had mutation and p value was 0.172. Among 23 vases who had recurrence 8 had SPINK1 mutation and p value was 0.244.

Table 2: Correlation of PRSS1 and SPINK1 mutation with parameters

Factors	Prss1 Mutation		Spink1 Mutation	
	Positive	Negative	Positive	Negative
Age In Years				
< 30	10	0	5	5
31-40	12	0	2	10
41-50	16	0	8	8
>50	4	0	3	1
Sex				
Male	29	0	11	18
Female	13	0	7	6
Ca 19.9 Levels				
More Than 37	4	0	3	1
Less Than 37	38	0	15	23
Recurrence				
Yes	23	0	8	15
No	19	0	10	9

Discussion

Chronic pancreatitis in adults is defined as a relapsing or continuing inflammatory disease of the pancreas characterized by irreversible morphological changes, upper abdominal pain and, in some patients, permanent impairment of exocrine function, endocrine function, or both. The pathogenesis of chronic pancreatitis is now considered a complex, multifactorial process. There is strong evidence that individual genetic susceptibility contributes significantly to disease development.

We did this study in 42 patients of chronic pancreatitis, among this 10 patients were less than 30 years, and 12 patients were in 31-40 years range, 16 patients in 41-50 year range and rest 4 patients above 50 years of age. In our study 29 patients were male and 13 patients were female.

In our study population abdominal pain was the main complaint in all patients. Pain was most common in epigastric region; mostly it was dull aching in nature. Most of patients had radiation to back and pain responded to analgesics. In our study population of 42 patients 36 patients had gone to hospital in past one year at least once for getting treated for pain. The main symptom of CP is pain; however, it is highly variable in character, frequency, and severity. [13]

Loss of appetite (n=28) and loss of weight (n=24) is the next common feature among our study participants. Anorexia, vomiting and steatorrhea were present in few cases in our study and jaundice and melena was also seen.

Diabetes (n=14) was most common comorbidity associated in patients included in our study group. Two patients had TB. 24 patients were alcoholic and 23 patients were smoker in our study group. 2 patients were HbsAg positive.

In our study population, no patients had family history of pancreatitis. Surgery required in 3 patients in our study. We analyzed recurrence and in our study 23 out of 42 patients had recurrence of episodes. Also we analysed CA 19.9 levels. Values above 37 were taken as positive and 4 among 42 patients in our study had levels in positive range.

We next did single nucleotide polymorphism of two genes in our study –PRSS1 and SPINK1. In our study heterozygous PRSS1 – positive for mutation was present in all patients in our study (100%). SPINK1 mutation was positive in 18 patients (43%) in our study. he most relevant genes include the cationic trypsinogen gene (PRSS1), the pancreatic secretory trypsin inhibitor gene (SPINK1), the cystic fibrosis transmembrane conductance regulator gene (CFTR), the chymotrypsinogen gene (CTRC), and the carboxy peptidase A1 gene (CPA1). These major genetic susceptibility factors were identified as candidate genes linked to intrapancreatic trypsin activity regulation within the pancreas or reduced ductal fluid flow. Within the "trypsin-dependent pathological pathway" variants in susceptibility genes PRSS1, SPINK1, and CTRC increase pancreatitis risk by promoting harmful trypsinogen activation or by impairing protective trypsinogen degradation and/or trypsin inhibition. Activation of PRSS1 trypsinogen to active trypsin within the pancreas is responsible for disease onset and progression. Protective mechanisms to control trypsinogen activation include trypsin inhibition by SPINK1 and trypsinogen degradation by chymotrypsin C (CTRC) and trypsin. SPINK1 mutations are found in 1–3% of the general population, [14] and idiopathic chronic pancreatitis is strongly associated with the common SPINK1 high-risk genotype N34S. [15] There may be a small effect of N34S in alcohol-related chronic pancreatitis patients, suggesting that alcohol's primary effects are driven through a non-trypsin related pathway.

We further analyzed this SNP results with various related factors. To start with Age distribution, As PRSS1 mutation was present in all cases, the above normal distribution was present in same way, while among 18 SPINK1 mutation cases most of cases were in 41-50 years age group. There was no significant relation between age and SPINK1 mutation with p value of 0.133. On average, irrespective of gender, those with SPINK-1 polymorphisms who develop pancreatitis do so at a younger age (mid 20's) when compared to those without it (mid 30's). Mutations do not follow autosomal dominant or recessive patterns and heterozygotes and homozygotes display similar rates of disease process, making the mutation a disease modifier rather than a direct cause.

Moving on to gender distribution, among 18 patients having SPINK1 mutation 11 were male and rest 7 was female and this was not statistically significant with a p value of 0.335. This is similar to results of Koziel D et al. There were no significant differences in frequencies of SPINK1, and PSSR1 mutations when comparing the male and female patients. [16] In our study population of 42 patients and only 4 had increased CA 19.9 levels and among which 3 had mutation and p value was 0.172. Among 23 cases that had recurrence 8 had SPINK1 mutation and p value was 0.244. Recent reports from India [17] and Japan [18] indicated that SPINK1 mutations confer strong genetic susceptibility to developing CP, but alone do not cause the disease. Some SPINK1 mutations alter peptide expression or binding affinity, though the disease-causing biochemical defect of the N34S mutation remains unknown.

Mutations are associated with recurrent acute and chronic pancreatitis since dysfunctional gene can result in retention of zymogens that can become active and result in pancreatitis [19]. Kumar et al. (2016) sought to characterize and identify risk factors associated with acute recurrent pancreatitis (ARP) and CP in childhood in a multinational cross-sectional study (INSPPIRE). [20]

The authors analyzed 301 children with ARP or CP. They found that "At least 1 gene mutation in pancreatitis-related genes was found in 48% of patients with acute recurrent pancreatitis vs 73% of patients with chronic pancreatitis. Children with PRSS1 or SPINK1 mutations were more likely to present with chronic pancreatitis.

Conclusion

Mutation of PRSS1 and SPINK1 may predispose patients to acute pancreatitis, especially in those abusing alcohol, and may promote a more severe course of the disease. Having an idea which polymorphisms confer susceptibility to acute pancreatitis, and which polymorphisms modify the immunological response to acute and recurrent acute pancreatitis that lead to chronic pancreatitis will also be important in determining the timing of genetic testing and subsequent intervention. Genetic testing in future will help in avoiding progress to Chronic or recurrence in pancreatitis patient by taking rational preventative measures.

Limitation of our study: Our study was done in shorter period as a cross sectional observational study in a small number of patients. Doing the study in a larger population as a multicentric study will show more light on probable impact of doing genetic testing in pancreatitis patient.

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