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Original Article

FREQUENCY OF GENETIC MUTATION OF CATIONIC TRYPSINOGEN (PRSS1) GENE IN PAEDIATRIC PATIENTS WITH PANCREATITIS ATTENDING A TERTIARY CARE HOSPITAL IN BANGLADESH

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Key words:
AP=acute pancreatitis, ARP= acute recurrent pancreatitis, CP= chronic pancreatitis, ICP= idiopathic chronic Pancreatitis, PRSS1= Cationic Trypsinogen Protease serine 1, CFTR= Cystic Fibrosis Transmembrane conductance Regulator.

Abstract

Introduction: *Pancreatitis is a global health problem. Up to 10-25% of patients who have no clear risk factors and are classified as idiopathic chronic pancreatitis (ICP). The pathogenesis of ICP is poorly understood. During last two decades it has become acceptable that development of pancreatitis requires a combination of genetic, environmental, structural or toxic insult. The aim of the study was to observe the mutation in PRSS1 gene in pediatric patients with pancreatitis. Methods: It was a cross-sectional descriptive study performed at the department of Paediatric Gastroenterology and Nutrition, BMU, Dhaka, Bangladesh from May 2019 to October 2020. Results: Out of 18 paediatric patients with pancreatitis 12, (66.7%) were female and six (33.3%) males. The mean age of the patients was 12.1 (±3.3) years and the mean age at first presentation was 8.7 year. Abdominal pain (100%) was the most common presenting feature. In the current study, in three (17%) patients PRSS1 gene mutation were observed while in most (15, 83%) of the cases such mutations were absent. In our study, a missense mutation G>A, was found that causes alteration of amino acid from Histidine to Arginine at 122 codon of PRSS1 gene and this mutation was pathogenic. There was one (33.3%) patient each with pancreatic duct dilatation, biliary sludge and pancreatic calcification had PRSS1 mutation. On etiological distribution, idiopathic and biliary tract abnormality showed statistical significance (p<0.05). Conclusion: In the current study, out of 18 pancreatic patients PRSS1 gene mutation was observed in three (17%) patients. These three patients with PRSS1 genetic mutation had pancreatic duct dilatation, biliary sludge and pancreatic calcification respectively.*

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Introduction

Pancreatitis is defined as the histological presence of inflammation within the parenchyma of the pancreas. The incidence of acute pancreatitis in the pediatric population has increased during the last two

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decades, ranging from 3.6 to 13.2 cases per 100,000 children, with a mortality rate between 4% and 10%.[1-3] Overall ARP is reported in 15–35% of children following an initial attack of AP and CP is estimated as ~0.5 per 100,000 persons per year.[4,5] Early onset chronic pancreatitis and idiopathic recurrent acute pancreatitis in the absence of any other established risk factors might result from genetic mutations. The first reported associated mutation was identified in the cationic trypsinogen gene (PRSS1) on chromosome 7. Additional mutations that may contribute are found in the serine protease inhibitor Kazal type 1, the CFTR gene and the chymotrypsinogen C gene. Mutations in these latter genes were responsible for pancreatitis that are initially classified as idiopathic chronic or idiopathic acute pancreatitis, although PRSS1 mutations have also been seen in non-hereditary cases. These mutations may have an additive effect, increasing individual susceptibility to pancreatitis.[6]

Cationic trypsin (PRSS1), the most abundant isoform of trypsin secreted by the pancreas. The role of cationic trypsin is to convert inactive pancreatic zymogens secreted by the pancreas into active digestive enzymes in the duodenum when stimulated by food. Premature conversion of trypsinogen to trypsin leads to premature activation of these digestive enzymes before excretion from the pancreas, which in turn leads to autodigestion of the parenchyma, leading to the inflammation and damage that manifests clinically as pancreatitis.[7] Genetic mutations in the PRSS1 gene may interfere with these defense mechanisms, ultimately leading to clinical pancreatitis. Chronic pancreatitis increases the risk of pancreatic cancer and hereditary pancreatitis has an estimated cumulative risk of pancreatic cancer in 40% .[6] The cumulative risk of exocrine insufficiency (60.2%) and diabetes (68.6%), is higher in HP than in other forms of pancreatitis.[8]

Identification of such mutation can help in genetic counseling, specific screening strategies for pancreatic cancer, lifestyle recommendations and modifications to prevent further attack of Pancreatitis. In addition, the identification of these gene mutations will decrease the incidence and prevalence of idiopathic pancreatitis as many of HP patients have been initially misdiagnosed as IP.

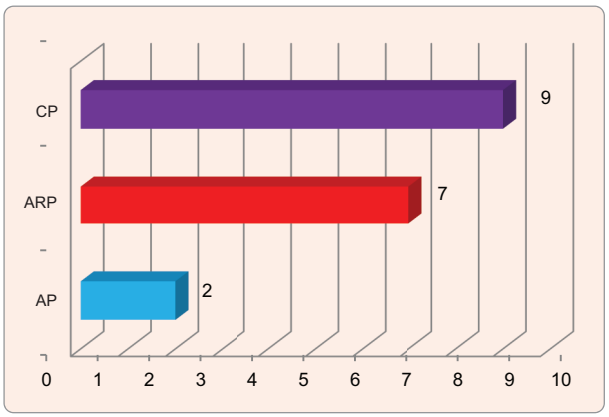
To the best of knowledge, in Bangladesh no study has been carried out so far to find out the genetic mutation of pancreatitis in children. As PRSS1 mutation was the most common mutation associated with pancreatitis and also due to COVID pandemic situation (less patient availability) and financial constraint (multiple genetic mutation could not be done) this study has been carried out to identify the presence of PRSS1 gene mutation in pancreatitis in children in a tertiary care center.

Materials and Methods

It was a cross-sectional descriptive study, was carried out at the Department of Paediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from May 2019 to October 2020. 18 children of either gender, age less than 18 years was selected who attended at the Department of Paediatric Gastroenterology and Nutrition, BSMMU with the diagnosis of pancreatitis or diagnosed after admission as pancreatitis were selected for the study. Any life-threatening complications of pancreatitis e.g. shock, systemic inflammatory response syndrome, etc and Patients with abdominal pain due to other known organic causes e.g. constipation, ureteric stone, etc were excluded from the study. After taking informed written consent from the guardian the studied populations were divided into three groups of pancreatitis according to INSPPIRE criteria as AP, ARP and CP. Data was collected in a structured questionnaire. After cleaning and editing, all the relevant data were compiled on a master chart. Statistical analysis of the results was obtained by SPSS for Windows (IBM SPSS Statistics for Windows, version 25.0, Armonk, NY, IBM Corp). Chromas 2.6.6 software had been used to analyze the mutational hotspots of PRSS1 gene by Sanger DNA sequencing. Categorical data were expressed as number and percentage and were compared via the Chi-squared test and Fischer's exact tests. Continuous data were expressed as mean ± SD and were compared by Student "t" test. Two tailed $p<0.05$ was considered as significant.

Results

The studied populations were diagnosed as acute, acute recurrent and chronic pancreatitis. Figure 1 showed majority (n-9, 50%) of children were suffering from chronic pancreatitis followed by acute recurrent pancreatitis (7, 39%) while 11.1% from acute pancreatitis (n=2).



AP= acute pancreatitis, ARP=acute recurrent pancreatitis and CP=chronic pancreatitis

Figure 1: *Bar diagram showing distribution of the patients by types of pancreatitis*

The study showed most of the patients were in the above 10 years age group in both ARP (n=6) and CP (n=7) cases. Three patients (16.7%) were from 6-10 years age group while two patients were from ≤ 5 year age group (Figure 2).

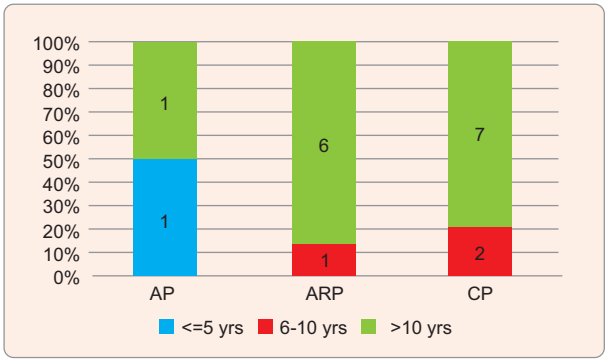


Figure 2: *Age distribution of the studied subjects (n-18)*

The study showed that the mean age of the patients was 12.1 (±3.3) years. Age range was from 3-16 years. Maximum number of patients were from age group e" 10 year (14, 77.8%) followed by 6–10-year age group (3, 16.7%). (Figure-3)

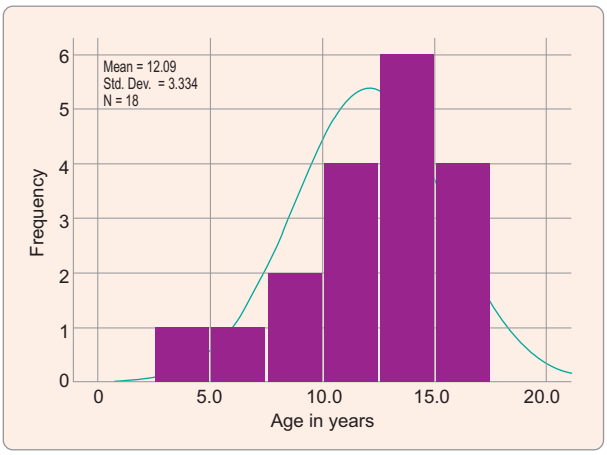


Figure 3: *Bar diagram showing age group distribution of the patients (n-18)*

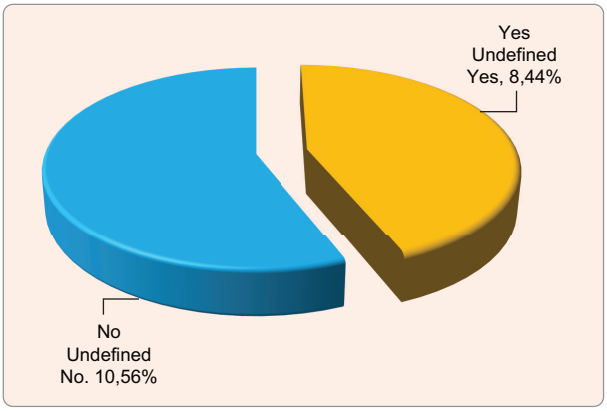


Figure 4: *Pie diagram showing excess fatty food intake (n-18)*

The study showed that two-third (n=12, 66.7%) respondents were female. It was also shown that numbers of female were higher in ARP and CP cases and no female in AP cases. (Figure 4).

Table –I showed that the mean age of the patients at current presentation was 12.1 years. The mean for AP patients (9.4 years) was slightly lower than that of the ARP (12.8 years) and CP (12.1 years) patients

Table-I

Distribution of patients by age at current presentation and age at 1st episode of pain

Variables	Total(n=18) Mean(±SD)	Diagnosis AP(n=2) Mean(±SD)	ARP(n=7) Mean(±SD)	CP(n=9) Mean(±SD)	*p*-value*
Age of current presentation	12.1 (3.3)	9.4 (7.9)	12.8 (2.6)	12.1 (2.9)	0.463
Age at 1st episode	8.9 (2.6)	9.4 (7.9)	9.1(1.9)	8.7 (1.7)	0.930

*ANOVA=Analysis of variance
p-value ≤ 0.05 is considered significant

but these differences were statistically not significant (p>0.05). The mean age of the patients at 1st episode of pain was 8.9 years. The mean age at 1st episode of pain for AP, ARP and CP patients were 9.4, 9.1 and 8.7 years respectively. Statistically, these differences were not significant (p>0.05)

Distribution of patients by presenting symptoms is presented in Table II. In almost all patients except one (17, 94.4%), vomiting was present. Beside this, nine (50.0%) subjects had loss of appetite and two (11.1%) children had abdominal distension. Only one (5.6%) child of AP group had fever. None of these differences was statistically significant (p<0.05).

This study showed that (Figure 5) eight patients (44%) had the habit of excess fatty food intake while remaining 10 patients (56%) had not.

Distribution of patients by signs is presented in Table III. In all (18, 100%) subjects abdominal tenderness was present. Anaemia was found in six subjects (33.3%); more common in AP (50%) and CP (33.3%) cases than ARP (28.6%) cases. Jaundice was present in three patients (16.7%); mostly in CP cases (22.2%). Dehydration was present in one patient (5.6%). None of these differences was statistically significant (p<0.05).

Table IV showed that abdominal pain was the most common presenting feature in pancreatitis. All the 18 patients of study had abdominal pain. Among them, nine (50%) subjects experienced dull type of pain, CP cases experienced more this type of pain than ARP or AP cases. Sharp and diffuse types of pain were experienced by four (22.2%) patients and CP cases reported most. These differences were statistically not significant (p>0.05). Regarding location, more than half (55.6%) of the subjects experienced epigastric pain. Beside these, five (27.8%) subjects reported umbilical pain and in one case pain in right hypochondriac region. But statistically the difference was not significant between AP, ARP and CP cases regarding location of pain.

Distribution of patients by imaging findings is presented in Table V. Pancreatic duct dilatation was found in 14 subjects (77.7%); mostly found in AP (100%) and CP (88.9%) cases. Pancreatic calcification was found in six (33.3%) patients and all of them were CP cases. This difference was statistically significant

Table II

Distribution of patients by presenting symptoms

Variables		Diagnosis			p-value*
	Total(n=18)	AP(n=2)	ARP(n=7)	CP(n=9)	
Vomiting	17 (94.4)	2 (100.0)	7 (100.0)	8 (88.9)	1.00
Loss of appetite	9 (50.0)	1 (50.0)	3 (42.9)	5 (55.6)	0.664
Abdominal distension	2 (44.4)	0 (0.0)	1 (14.3)	1 (11.1)	1.00
Fever	1 (5.6)	1 (50.0)	0 (0.0)	0 (0.0)	0.111

* Fishers' Exact test; percentages are given in parenthesis;
p-value d—0.05 is considered significant

Table-III

Distribution of patients by signs

Variables		Diagnosis			p-value*
	Total(n=18)	AP(n=2)	ARP(n=7)	CP(n=9)	
Abdominal tenderness	18 (100.0)	2 (100.0)	7 (100.0)	9 (100.0)	1.00
Anaemia	6 (33.3)	1 (50.0)	2 (28.6)	3 (33.3)	0.471
Jaundice	3 (16.7)	0 (0.0)	1 (14.3)	2 (22.2)	0.611
Dehydration	1 (5.6)	1 (50.0)	0 (0.0)	0 (0.0)	0.111

* Fishers' Exact test; percentages are given in parenthesis;
p-value d—0.05 is considered significant

(p<0.05). Pancreatic duct calculi were noted in four (22.2%) patients. Pancreatic pseudocyst was noted in one (5.6%) patient.

Means of various laboratory parameters are compared in the Table VI. Mean haemoglobin level was 11.7 g/dL. No significant difference was observed in mean haemoglobin levels across different groups by ANOVA test. This was also applicable for total count, platelet count and haematocrit values.

Means of various biochemical parameters are compared in the Table VII. Mean serum lipase level differed significantly across groups. It was much higher in ARP (3085.14 (4487.79) than other categories. In AP patients mean serum sodium level (131.0 SD± 2.83) was significantly less than ARP or CP patients.

Majority (55.6%) of the patients had showed (Table VIII) swollen pancreas which was more common in ARP than CP. Shrunken pancreas was found only in CP patients. Gall bladder sludge was found in three cases with CP and in two patients with ARP. Ascites was only found in ARP cases (n=4, 57.1%). Differences of GB sludge and ascites were statistically significant (p<0.05).

The bar diagram (Figure 5) shows PRSS1 gene mutation in the patients suffering from different types

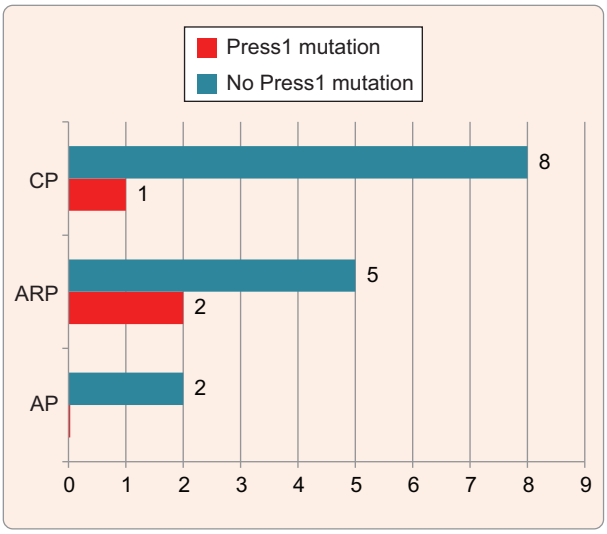


Figure 5: *Bar diagram showing PRSS1 gene mutation (n-18)*

Table-IV

Distribution of patients by character and location of pain (n-18)

Characteristics of pain			Diagnosis		*p*-value
	Total (n=18)	AP(n=2)	ARP(n=7)	CP(n=9)	
Dull	9 (50.0)	1 (50.0)	3 (42.9)	5 (55.6)	1.00
Sharp	4 (22.2)	1 (50.0)	1 (5.6)	2 (22.2)	
Diffuse	4 (22.2)	0 (0.0)	2 (28.6)	2 (22.2)	
LocalizedLocation of pain	1 (5.6)	0 (0.0)	0 (0.0)	1 (11.1)	
Epigastric	10 (55.6)	1 (50.0)	4 (57.1)	5 (56.5)	1.00
Umbilical	5 (27.8)	0 (0.0)	2 (28.6)	3 (33.3)	
Rt hypo-chondriac	1 (5.6)	0 (10.0)	1 (14.3)	(0.0)	

*Results were expressed in frequencies and percentage
* Fishers' Exact test; *p*-value ≤0.05 is considered significant
Zero indicates no cases in corresponding group

Table-V

Distribution of patients by imaging findings

Variables			Diagnosis		*p*-value*
	Total(n=18)	AP(n=2)	ARP(n=7)	CP(n=9)	
Pancreatic duct dilatation	14 (77.7)	2 (100.0)	4 (57.1)	8 (88.9)	0.273
Pancreatic calcification	6 (33.3)	0 (0.0)	0 (0.0)	6 (66.7)	0.009*
Pancreatic duct calculi	4 (22.2)	0 (0.0)	0 (0.0)	4 (44.4)	0.115
Pancreatic pseudocyst	1 (5.6)	0 (0.0)	0 (0.0)	1 (11.1)	1.00

* Fishers' Exact test; percentages are given in parenthesis;
p-value ≤0.05 is considered significant

Table-VI

Comparison of haematological parameters in pancreatitis

Variables	Diagnosis				*p*-value
	Total(n=18) Mean(±SD)	AP(n=2) Mean(±SD)	ARP(n=7) Mean(±SD)	CP(n=9) Mean(±SD)	
Haemoglobin(g/dL)	11.7 (1.5)	11.4 (2.6)	12.3 (1.4)	11.4 (1.4)	0.481
Total count(K/mm^3)	11.1 (3.9)	11.8 (1.1)	13.3 (2.9)	9.3 (4.2)	0.121
Platelet count(lac/mm^3)	3.1 (0.7)	3.3 (0.4)	2.9 (0.6)	3.2 (0.8)	0.682
Haematocrit(%)	36.1 (3.7)	36.5 (9.1)	36.6 (3.2)	35.7 (3.4)	0.121

ANOVA=Analysis of variance

p-value d—0.05 is considered significant

Table-VII

Distribution of patients by biochemical parameters

Variables	Diagnosis				*p*-value
	Total(n=18) Mean(±SD)	AP(n=2) Mean(±SD)	ARP(n=7) Mean(±SD)	CP(n=9) Mean(±SD)	
Serum creatinine (mg/dL)	0.52 (0.07)	0.48 (0.03)	0.53 (0.09)	0.53 (0.06)	0.640
RBS (mmol/L)	5.63 (1.22)	3.80 (1.13)	5.92 (1.16)	5.80 (1.03)	0.071
Serum lipase (U/L)	1532.61 (2975.95)	423.00 (445.47)	3085.14 (4487.79)	571.67 (483.61)	0.218
Serum amylase (U/L)	982.17(1228.58)	1463.50 (65.76)	1819.29 (1574.53)	224.11 (173.21)	0.019
Serum sodium (mmol/L)	137.22 (3.74)	131.00 (2.83)	138.43 (1.81)	137.67 (3.87)	0.029
Serum potassium (mmol/L)	4.13 (0.30)	3.97 (0.18)	4.09 (0.28)	4.20 (0.34)	0.605
Serum calcium (mg/dL)	9.36 (0.62)	9.10 (0.01)	9.15 (0.62)	9.59 (0.64)	0.310
Serum triglyceride (mg/dL)	212.78 (390.04)	117.00 (79.19)	348.14 (623.21)	128.78 (70.23)	0.530

ANOVA=Analysis of variance

p-value $\leq$ 0.05 is considered significant

Table-VIII

Sonographic findings of studied population (n-18)

Sonographic findings	Total(n=18) n(%)	AP(n=2) n (%)	ARP(n=7) n (%)	CP(n=9) n (%)	*p*-value*
Swollen pancreas	10 (55.6)	2 (100.0)	5 (71.4)	3 (33.3)	0.214
Shrunken pancreas	3 (16.7)	0 (0.0)	0 (0.0)	3 (33.3)	0.305
Panc. Calcification	3 (16.7)	0 (0.0)	0 (0.0)	3 (33.3)	0.305
GB sludge	5 (27.8)	2 (100.0)	3 (42.9)	0 (0.0)	**0.037***
Dilated CBD	0 (0)	0 (0.0)	1 (14.3)	1 (11.1)	1.00
Cholelithiasis	1 (5.6)	0 (0.0)	1 (14.3)	0 (0.0)	0.500
Ascites	4 (22.2)	0 (0.0)	4 (57.1)	0 (0.0)	**0.018***

Results were expressed in frequencies and percentage

Fisher's Exact test was done as a test of significance

p-value d"0.05 is considered significant

Zero indicates no cases in corresponding group

of pancreatitis. Out of 18 patients PRSS1 mutation was noted in three patients where two ARP cases and one CP showed PRSS1 mutation. No mutation was found among AP group.

Table IX showed that c.365G>A (p. Arg122His) was detected in three patients out of eighteen patients with pancreatitis. c.365G>A (p. Arg122His) means amino acid arginine is replaced by histidine at 122

codon of PRSS1 gene of 7th chromosome. All three patients had heterozygous type of mutation.

Figure 6 showed normal chromatogram of a patient where G stands for guanine, T stands for thiamine, C for cytosine and A for adenine and they are represented in black, red, blue and green colour respectively.

Figure 7: showed site (aerrow) of heterozygous PRSS1mutation (pArg122His) at 122 position in exon 3 of chromosome 7 for pancreatitis. Here site of heterozygous mutation means amino acid substitution e.g. Arginine is replaced by Histidine in a single allele.

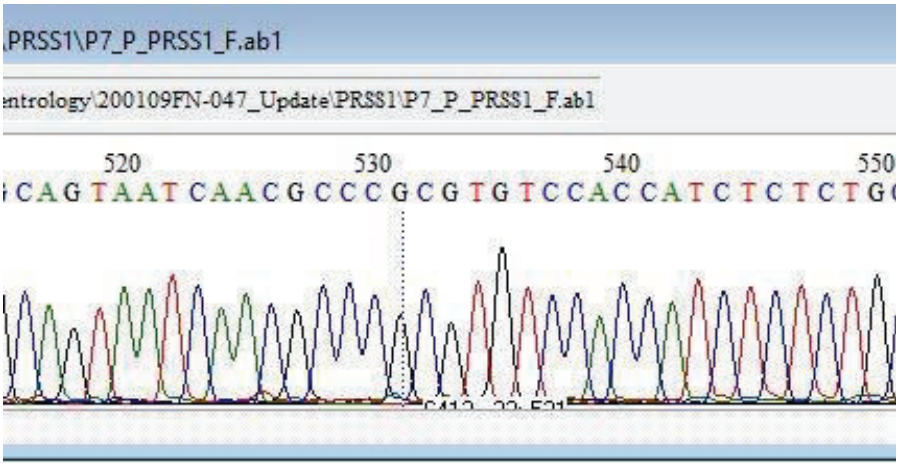
In Table X studied population with different types of pancreatitis, PRSS1 mutation was observed. Among them mutations were found more among ARP (28.6%) patients than CP patients (11.1%).

Table XI showed that two female patients, suffering from acute recurrent pancreatitis, had PRSS1 mutation while one female patient with chronic pancreatitis had such genetic mutation. This difference was not statistically significant (p>0.05). No male patients in this study had mutation.

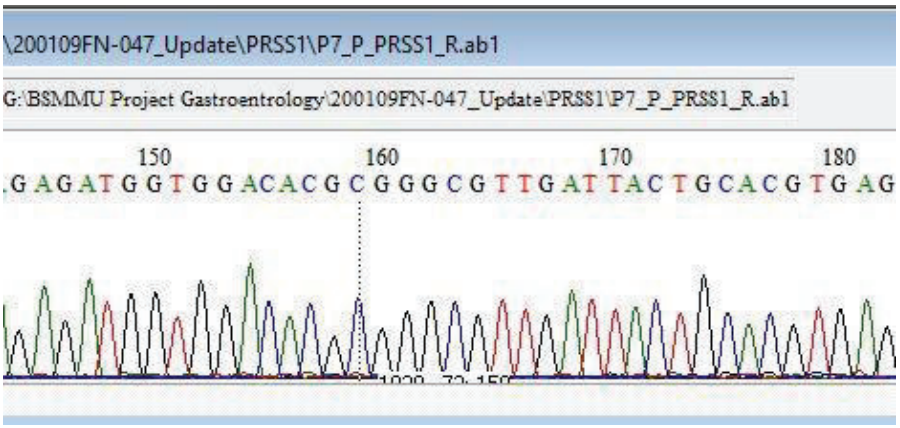
Table-IX

Distribution of PRSS1 gene Mutation detected in exon 3

Gene name	Position	Allele change	Genotype	Mutation type	Amino acid change
PRSS 1	chr7:142751938rs 17107315	G>A	Heterozygous	Missence	p.Arg122His
	chr7:142751938rs 17107315	G>A	Heterozygous	Missence	p.Arg122His
	chr7:142751938rs 17107315	G>A	Heterozygous	Missence	p.Arg122His

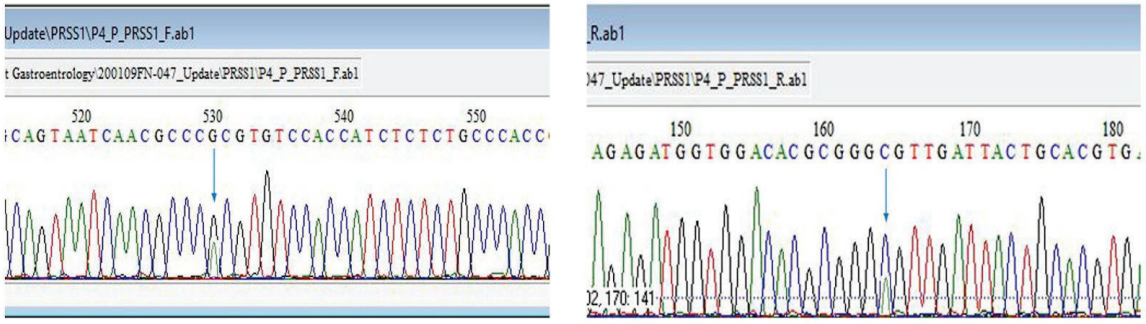


Forward Primer

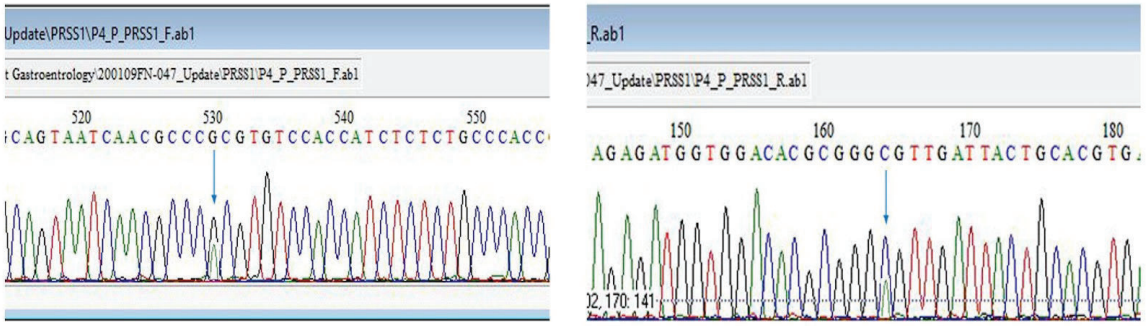


Reverse Primer

Figure 6: *Chromosome 7 analysis of a normal subject at exon 3*



Forward Primer



Reverse Primer

Figure 7: *Chromatographic changes showing PRSS1 genetic mutation*

Table-X

Distribution of PRSS1 by acute, chronic and acute recurrent pancreatitis

Type of pancreatitis	PRSS 1 mutation	
	Presentn (%)	Absentn (%)
AP (n-2)	0 (0.0)	0 (0.0)
ARP (n-7)	2 (28.6)	5 (71.4)
CP (n-9)	1 (11.1)	8 (88.9)

*Results were expressed in frequencies and percentage

Table XI

Distribution of PRSS1 mutation according to gender in acute, chronic and acute recurrent pancreatitis

PRSS1 mutation	AP	ARP	CP	*p*-value
Female (n=12)	0 (0.0)	2 (16.7)	1 (8.3)	0.523
Male (n=6)	0 (0.0)	0 (0.0)	0 (0.0)	

*Results were expressed in frequencies and percentage

Fisher's Exact test was done as a test of significance
p-value ≤0.05 is considered significant
Zero indicates no cases in corresponding group

Table –XII showed that one (33.3%) patient with PRSS gene mutation had pancreatic duct dilation but two (66.7%) patients with gene mutation did not have such dilation. Statistically this difference was not significant (p>0.05).

Table XII

Association between PRSS1 gene mutation and pancreatic duct dilatation.

Pancreatic duct dilatation	PRSS1 gene mutation		Fisher's Exact test	*p*-value
	Present	Absent	Present	
	1 (33.3)	13 (86.7)		
Absent	2 (66.7)	2 (13.3)	4.11	0.108
Total	3 (100.0)	15 (100.0)		

Results were expressed in frequencies and percentage
Fisher's Exact test was done as a test of significance
p-value ≤0.05 is considered significant
Zero indicates no cases in corresponding group

Table XIII showed one (33.3%) patient with PRSS gene mutation had pancreatic duct calculi but two (66.7%) patients with gene mutation did not have calculi. Statistically this difference was not significant (p>0.05).

All three (100.0%) patients with gene mutation did not have pancreatic pseudocyst. Statistically this difference was not significant (p>0.05). (Table XIV)

Table XV showed One (33.3%) patient with PRSS1 gene mutation had pancreatic calcification and two (66.7%) patients with gene mutation did not have pancreatic calcification. Statistically this difference was not significant (p>0.05).

Table XVI showed two (66.7%) patients with PRSS1 gene mutation had features of acute pancreatitis on abdominal USG while one (33.3%) patient with gene mutation had ultrasonographic features of chronic pancreatitis. However, statistically this difference was not significant (p>0.05).

One patient suffering from biliary sludge found to have PRSS1 gene mutation. In four patients with biliary sludge had no PRSS1 mutation was noted. There was one patient each with choledocal cyst and pancreas divisum without genetic mutation. However, these differences were statistically not significant (p>0.05). (Table XVII)

Etiological findings showed that (Table XVIII) in 33.3% cases cause of pancreatitis was not known. Among possible known causes, biliary tract abnormality (n=6) ranked top on the list. Other causes were PRSS1mutation (3), metabolic e.g., hyper-tryglyceridemia (1), choledocal cyst (1), trauma (1) and pancreas divisum (1). Idiopathic and biliary tract abnormality showed statistically significant (p<0.05) difference between different types of pancreatitis (i.e., AP, ARP & CP).

Table XIII

Association between PRSS1 gene mutation and pancreatic duct calculi.

Pancreatic duct calculi	PRSS1 gene mutation		Fisher's Exact test	*p*-value
	Present	Absent		
Present	1 (33.3)	3 (20.0)		
Absent	2 (66.7)	12 (80.0)	0.257	1.00
Total	3 (100.0)	15 (100.0)		

Results were expressed in frequencies and percentage
Fisher's Exact test was done as a test of significance
p-value ≤0.05 is considered significant

Table-XIV

Association between PRSS1 gene mutation and pancreatic pseudocyst.

Pancreatic pseudocyst	PRSS1 gene mutation		Fisher's Exact test	*p*-value
	Present	Absent		
Present	0 (0.0)	1 (6.7)		
Absent	3 (100.0)	14 (93.3)	0.212	1.00
Total	3 (100.0)	15 (100.0)		

Results were expressed in frequencies and percentage
Fisher's Exact test was done as a test of significance
p-value ≤0.05 is considered significant
Zero indicates no cases in corresponding group

Table XV

Association between PRSS gene mutation and pancreatic calcification.

Pancreatic calcification	PRSS1 gene mutation		Fisher's Exact test	*p*-value
	Present	Absent		
Present	1 (33.3)	5 (33.3)		
Absent	2 (66.7)	10 (66.7)	0.001	1.00
Total	3 (100.0)	15 (100.0)		

Results were expressed in frequencies and percentage
Fisher's Exact test was done as a test of significance
p-value ≤0.05 is considered significant

Table-XVI

Association between PRSS1 gene mutation and abdominal USG findings.

Abdominal USG findings	PRSS1 gene mutation		Fisher's Exact test	*p*-value
	Present	Absent		
	PRSS1 gene mutation	Fisher's Exact test	*p*-value	
	Present	Absent		
AP	2 (66.7)	8 (53.3)		
CP	1 (33.3)	7 (46.7)	0.180	1.00
Total	3 (100.0)	15 (100.0)		

Results were expressed in frequencies and percentage
Fisher's Exact test was done as a test of significance
p-value d"0.05 is considered significant

Table-XVII

Distribution of the subjects by pancreaticobiliary disorder and PRSS1 gene mutation

Pancreatic -biliary disorder	PRSS1 gene mutation		Fisher's Exact test	*p*-value
	Present (n=3)	Absent (n=15)		
Choledocal cyst (n-1)	0 (0.0)	1 (6.7)	1.534	1.00
Bliliary sludge (n-5)	1 (33.3)	4 (26.7)		
Pancreas divisum (n-1)	0 (0.0)	1 (6.7)		

Results were expressed in frequencies and percentage
Fisher's Exact test was done as a test of significance
p-value d"0.05 is considered significant
Zero indicates no cases in corresponding group

Table-XVIII

Etiological distribution of the studied population

Etiology	Total (n=18) n (%)	AP (n=2) n (%)	ARP (n=7) n (%)	CP (n=9) n (%)	*p*-value*
Idiopathic (n-6)	6 (33.3)	2 (100)	3 (42.9)	1 (11.1)	0.025*
Biliary (n-6)	6 (33.3)	0 (0.0)	3 (42.9)	3 (33.3)	0.037*
Metabolic (high TG) (n-1)	1(5.6)	0 (0.0)	1 (14.3)	0 (0.0)	1.00
Choledocal cyst (n-1)	1(5.6)	0 (0.0)	0 (0.0)	1 (11.1)	1.00
Pancreas divisum (n-1)	1(5.6)	0 (0.0)	0 (0.0)	1 (11.1)	1.00
Trauma (n-1)	1(5.6)	0 (0.0)	0 (0.0)	1 (11.1)	1.00
PRSS1 mutation(n-3)	3(16.7)	0 (0.0)	2 (28.7)	1 (11.1)	0.185

Results were expressed in frequencies and percentage
Fisher's Exact test was done as a test of significance
p-value ≤0.05 is considered significant
Zero indicates no cases in corresponding group

Discussion

The present study has been carried out to determine the frequency of PRSS1 gene mutation in pediatric patients with pancreatitis in a tertiary care centre in Bangladesh, as well as to document the frequency of PRSS1 gene mutation in pancreatic duct stenosis or dilation, pancreatic duct stones, pancreatic pseudocyst and pancreatic calcification. A total of 18

patients with pancreatitis fulfilling the diagnostic criteria were included in the study.

The etiological causes were diverse in pediatric pancreatitis. Etiological findings showed that in 33.3% cases cause of pancreatitis was not known. Most common known cause was biliary tract diseases (33.3%), it included calculi, choledochal cyst, and gall bladder sludge. PRSS1 gene mutation was found in 16.7% cases. Other causes were metabolic e.g. hypertryglyceridemia (5.6%) and pancreas divisum (5.6%). Statistically significant difference was noted between different types (i.e. AP, ARP & CP) and idiopathic and biliary causes (p<0.05). A study also reported unknown cause to be the most common (34%) etiology.[9] However, there were some other studies which showed different scenario. A study by found the primary cause to be traumatic (36.3%), second common cause was idiopathic pancreatitis (25.1%), followed by systemic diseases (22.2%), metabolic (5.8%), biliary diseases (5.4%), drugs (3.2%) and viral infections (2.2%).[10] Another study reported that biliary diseases (gallstones/sludge) was the most prevalent etiological cause (24.3%), followed by trauma (16.2%) and drug (10.8%).[11] It was found in a study that seventeen of the 32 patients presented with unknown etiologies and 15 patients presented with associated pancreaticobiliary disorders such as choledochal cysts (CDCs) (n =4), biliary sludge/ stones (n =6) and pancreas divisum (n =6) . Among 14 patients with CP, 8 patients were idiopathic and 6 patients presented with associated pancreaticobiliary disorders. Chronic pancreatitis was more common in pancreas divisum (n= 4) than in CDC (n=1) or biliary sludge/ stone (n=1).[12]

Factors that predispose children to recurrent attacks of AP and progression from ARP to CP are unknown. Although alcohol and smoking have long been recognized as major risk factors for ARP and CP in adults, they are uncommon in the pediatric age group.[13] Recent single-center study have identified several genetic risk factors in children with ARP or CP.[14,15] Other risk factors included obstructive, traumatic, infectious and metabolic causes.[16] In the current study, PRSS1mutation was identified only in 3 patients (16.7%) out of 18 patients. Due to lack of investigation facilities and due to financial constrain, all the known other genetic susceptibility factors for pancreatitis identified till date including polymorphisms/ mutations in genes namely cationic trypsinogen (PRSS1), chymotrypsin C (CTRC), Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) could not be performed in all patients. That may be the cause of increased percentage of idiopathic pancreatitis in this study.

The diagnosis of pancreatitis can be made with reasonable certainty on the basis of clinical, radiological and laboratory findings. Ultrasonography has been shown to have 80% accuracy in the diagnosis of pancreatitis, usually shows decreased echogenicity of the pancreas.[17] Abdominal ultrasonography enables visualization of findings associated with acute pancreatitis such as pancreatic enlargement, inflammatory changes around the pancreas and ascites which are useful in making a diagnosis of acute pancreatitis.

It is also useful in detecting biliary stones responsible for acute pancreatitis and differentiating acute pancreatitis from other abdominal diseases.[18] In this present study, abdominal ultrasonogram was carried out in all patients (100%), it aided in establishing the diagnosis and find out the etiology such as biliary tree abnormality which included pancreatic calcification (16.7%), calculi in gall bladder (5.6%) or gall bladder sludge (27.8%). Swollen pancreas was found in 55.6% cases among acute, chronic and acute recurrent pancreatitis. Enlarged and edematous pancreas are classic sonographic features of acute pancreatitis.[19] Ascites was also a significant finding among acute recurrent pancreatitis.

Pancreatic duct outflow obstruction is one of the most common causes of ARP because it induces transient or persistent intraductal hypertension. It may also occur when the obstruction involves the pancreaticobiliary junction, causing reflux of bile into the main pancreatic duct. Obstruction can occur from common bile duct stone disease (including sludge and bile crystals), sphincter of Oddi dysfunction (SOD), anatomical variants of the pancreatic ductal system or pancreaticobiliary junction, pancreatic duct calcification, choledochocele and lesions of the main pancreatic duct, either benign or malignant. In the current study, 27.8% patients had biliary sludge, among them 1 had PRSS1 mutation and rest had no mutation and each patient with pancreatic duct stone (33.3%) and pancreatic calcification (33%) was found to have PRSS1 mutation. In contrast, in 2009, a study among 200 French patients with HP was carried out and it was found pseudocysts in 23% and calcifications in 61% patients.[8,20]

In the current study in three patients (17%) PRSS1 gene mutation were observed while in most of the cases (15, 83%) such mutation was absent. In our study a missense mutation G>A, was found that causes alteration of amino acid from Histidine for Arginine at 122 codon of PRSS1 gene. This mutation is pathogenic and was found in the patients of hereditary pancreatitis and tropical calcific pancreatitis. Our current data on the frequency of PRSS1 mutations were similar to those (17% vs 9–23%) of a previous study on children with CP or ICP. Moreover, A study ûrst showed PRSS1 gene mutations (3/30 cases at 3×A16 V) in German paediatric CP patients[21]. Thereafter, they showed that in a study of 96 unrelated CP children, PRSS1 gene mutations (5×A16 V, 1×N29I and 5×R122H) occurred in 11 (11.5%) patients. In a review of 164 unrelated children with CP, the frequency was reported to be 9.1% (n=15, 8×A16 V, 5×R122H and 2×N29I)[22]. PRSS1 gene mutations were detected in two (12.5%, 1×R122H and 1×A16 V) of 16 patients classiûed as having early-onset ICP in a Swiss study and in 11 (23.1%) of 52 children with CP (6×R122H, 4×R122C, 1×N29I) in a Polish study.[23,24] Heterozygous mutations of the PRSS1 gene commonly occurred in CP. The PRSS1 mutations seem to be one of the predisposing factors for ICP, irrespective of race. However, this frequency is different from most previous Asian studies, in which PRSS1 mutations were at a low frequency or even absent.[25,26]

A study showed that patients with mutations were more likely to have pancreatic duct stones or pancreatic calcification than those without such a mutation.[27] It has been well known that patients with pancreatic calcification were more severe than those without pancreatic calcification. Moreover, patients with CP have a markedly increased risk in developing pancreatic cancer compared to the general population and PRSS1 mutation may be a predictor for pancreatic cancer development in patients with CP[20]. Thus, CP patients with PRSS1 mutation should avoid any risk factors including alcohol and tobacco, be monitored for any signs or symptoms (pain, weight loss, jaundice and/or abdominal mass) or with serum markers and imaging examination for pancreatic cancer.

Conclusion

In this study, out of 18 pancreatic patients PRSS1 gene mutation were observed in three patients (17%) while in most of the cases (83%) such mutations were absent. A missense mutation G>A was found that caused alteration of amino acid from Histidine by Arginine at 122 codon of PRSS1 gene and this mutation was pathogenic. Pancreatic duct dilatation, biliary sludge and pancreatic calcification were found in three cases with PRSS1 mutation respectively.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The study was approved by the Institutional review board, BSMMU, Dhaka, Bangladesh.

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