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Alpha-1 antitrypsin deficiency and pancreatitis in children

Niedobór alfa1-antytrypsyny a zapalenie trzustki u dzieci

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Słowa kluczowe

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S u m m a r y

Introduction. Alpha-1 antitrypsin is one of the most important serum inhibitors of proteolytic enzymes such as trypsin, chymotrypsin and pancreatic elastase. There is a hypothesis that increased levels of pancreatic proteinases or a decrease in pancreatic antiproteinases can lead to pancreatitis.

Aim. To evaluate the significance of alpha-1 antitrypsin deficiency in children with chronic, acute recurrent and acute pancreatitis.

Material and methods. 83 children with chronic pancreatitis (CP), acute recurrent pancreatitis (ARP) and acute pancreatitis (AP) were enrolled into the study. Genotyping for E264V (PiS) and E342K (PiZ) variants of AAT was done. In 65% of patients (54/83) simultaneously with genetic testing, alpha-1 antitrypsin deficiency serum level was measured. In view of the results as shown below it was not necessary to use statistical analysis.

Results. Only in 1 of 83 patients alpha-1 antitrypsin deficiency was recognized. It was almost 13-years old, obese girl with CP, in which E342K mutation in one allele (PiZ heterozygote) and decreased alpha-1 antitrypsin serum concentration were found. In addition to severe AP episode, elevated levels of serum transaminases and hepatic steatosis in CT imaging and liver biopsy was observed.

Conclusions. In the present study, genetic analysis of the two most common alpha-1 antitrypsin deficiency alleles PiS and PiZ revealed no association between alpha-1 antitrypsin genotypes and development of CP, ARP or AP, which remains in line with the results of most previous studies. In conclusion, it seems that α1-antitrypsin is not involved in the pathogenesis of pancreatitis in children.

S t r e s z c z e n i e

Wstęp. Alfa1-antytrypsyna (AAT) jest jednym z najważniejszych krążących w osoczu inhibitorów enzymów proteolitycznych trzustki trypsyny, chymotrypsyny i elastazy. Od ponad stu lat istnieje teoria, że zaburzenie równowagi między proteazami oraz ich inhibitorami w miąższu trzustki prowadzi do jej samotrawienia i zapoczątkowania procesu zapalnego narządu. Istnieją badania i opisy przypadków, w których sugeruje się, że wspomniana dysproporcja między proteazami i ich inhibitorami u pacjentów z niedoborem AAT przyczynia się do rozwoju przewlekłego zapalenia trzustki (PZT).

Cel pracy. Retrospektywna ocena związku między niedoborem alfa1-antytrypsyny a występowaniem przewlekłego zapalenia trzustki, ostrego nawracającego zapalenia trzustki (ONZT) oraz ostrego zapalenia trzustki (OZT) u dzieci.

Materiał i metody. Do badania włączono 83 dzieci z PZT, ONZT i OZT, u których w przebiegu diagnostyki przyczyn choroby wykonano genotyp alfa1-antytrypsyny (warianty E264V (PiS) i E342K (PiZ)). U 65% pacjentów (54/83) jednocześnie z badaniem genetycznym oznaczono stężenie AAT w surowicy. W świetle wyników, które przedstawiono poniżej, nie było konieczne korzystanie z analizy statystycznej.

Wyniki. Niedobór alfa1-antytrypsyny rozpoznano jedynie u 1 z 83 pacjentów. Była to prawie 13-letnia, otyła dziewczynka z PZT, u której stwierdzono mutację E342K w jednym allelu (heterozygota PiZ) oraz obniżone stężenie AAT w surowicy. Poza PZT u pacjentki zaobserwowano hipertransaminazemię, stłuszczenie wątroby w badaniu tomografii komputerowej (TK) jamy brzusznej oraz w biopsji wątroby.

Wnioski. W naszej pracy, opierając się na badaniu genetycznym dwóch najczęstszych mutacji będących przyczyną niedoboru alfa-1 antytrypsyny, stwierdziliśmy brak bezpośredniego związku między niedoborem AAT a rozwojem PZT, ONZT lub OZT. Wyniki te są zgodne z większością do tej pory przeprowadzonych badań.

Konflikt interesów
Conflict of interest

Brak konfliktu interesów
None

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INTRODUCTION

Chronic pancreatitis (CP) is a rare disease in children, characterized by continuous structural and/or functional damage of the pancreas resulting from its progressive inflammation. The most common causes of chronic pancreatitis (CP) in children differ significantly from those reported in adults. Principal etiological factors of CP and recurrent acute pancreatitis (RAP) in pediatric patients include anatomic anomalies and gene mutations, lipid disorders and biliary tract diseases. A large percent of CP cases (30-40%) is still described as idiopathic (1-5).

For over a hundred years, there has been a theory that pancreatitis arises from premature activation of pancreatic zymogens to active enzymes within the pancreas parenchyma, leading to autodigestion and inflammatory process of the pancreas. One of the most common genetic mutations in children with CP are, among others, in the genes encoding cationic trypsinogen (Protease, Serine, 1 – *PRSS1*) and trypsinogen protease inhibitor type 1 (Serine Protease Inhibitor Kazal type 1 – *SPINK1*) (4-8). It is believed that these mutations lead to imbalance between the proteases and their inhibitors in the pancreas. In patients with idiopathic CP the prevalence of *SPINK1* mutation according to the various studies varies from 6.5% to almost 45% and it is much higher than the population frequency (3, 7-14). A heterozygous pathogenic variant *PRSS1*, which results in autosomal dominant inheritance of pancreatitis, is found in 9.1-11.5% children with CP (15, 16). Among patients with idiopathic CP (studies included both children and adults) the frequency of *PRSS1* ranges between 0% to 12.5% (17-21). According to a study by Oracz et al., in a group of over 200 polish children with CP, *SPINK1* mutation occurred in 20.2% of patients and *PRSS1* mutation was found in 10.6% of patients (4).

Alpha-1 antitrypsin deficiency (AAT) is one of the most important circulating inhibitor of pancreatic proteolytic enzymes – trypsin, chymotrypsin and elastase. Two frequent genetic defects in the alpha-1 antitrypsin deficiency gene are known: a glutamine to valine substitution at codon 264 in exon 5 (E264V) (PiS) (8-10) and a glutamine to lysine substitution at codon 342 in exon 7 (E342K) (PiZ) (22). In CP potential role of alpha-1 antitrypsin deficiency it is of particular interest because it may prevent pancreatic autodigestion by inhibiting the proteases activity. There are studies and case reports in which it is suggested that imbalance between proteases and their inhibitors in patients with alpha-1 antitrypsin deficiency contributes to the development of CP (23-25). However, in subsequent studies, authors found no association between AAT deficiency and the development of inflammation (26-29).

In view of the high prevalence of patients with *SPINK1* and *PRSS1* mutations and conflicting results of previous studies we decided to evaluate the association between alpha-1 antitrypsin deficiency and the development of CP, ARP and acute pancreatitis (AP) in a large single-centre group of paediatric patients.

AIM

The aim of this study was to evaluate the significance of alpha-1 antitrypsin deficiency in children with chronic, acute recurrent and acute pancreatitis.

MATERIAL AND METHODS

Patients with chronic pancreatitis (CP), acute recurrent pancreatitis (ARP) and acute pancreatitis (AP) were admitted to the Department of Gastroenterology, Hepatology, Feeding Disorders and Pediatrics of The Children's Memorial Health Institute between 2000 and 2011. They were enrolled for genetic and clinical investigation. A total of 83 Polish patients (age of disease onset 2.0-19.5 years, mean age 8.9 years, 41 female and 42 male) were studied. The cohort of patients included 65 CP patients (age of disease onset 2.0-19.5 years, mean age 8.5 years), 4 with ARP (age of disease onset 2.6-10.8 years, mean age 5.9 years) and 14 with AP (age of first AP episode 4.8-17.7 years, mean age 11.3). The diagnosis of AP was established by the presence of 2 of the 3 following criteria: abdominal pain consistent with the disease, serum amylase and/or lipase greater than three times the upper limit of normal, and/or characteristic findings from abdominal imaging. Criteria for ARP were defined as 3 or more episodes of acute pancreatitis without evidence of CP in imaging studies. Chronic pancreatitis was diagnosed as pancreatitis with evidence of CP in imaging studies according to the Cambridge classification system. A significant percentage of patients with CP have a genetic basis for their disorder – 25/83 (30%): 11 patients have gene *SPINK1* mutation, 10 patients – *PRSS1* mutation, 5 – *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) mutation and 4 – *CTRC* (Chymotrypsin C) mutation. In 4 patients coexisted two types of mutations. A positive family history of pancreatitis was found for 19 patients. For a group of patients, establishing of probable cause of pancreatitis was possible: hypertriglyceridemia (4 patients), anatomical anomalies as pancreas divisum, ansa pancreatica or double Santorini duct (11 patients), biliary disease (choledochocele, choledocholithiasis, cholelithiasis and primary sclerosing cholangitis) (7 patients), trauma (5 patients), autoimmune pancreatitis (1 patient). The baseline characteristics of studied group are shown in table 1. Thirty-eight patients (46%) were classified as idiopathic pancreatitis.

The patients were informed on the aims of the project, and their written consent form for molecular procedures was obtained. DNA was extracted from leukocytes of EDTA-anticoagulated blood according to standard protocols (30). Genotyping for E264V (PiS) and E342K (PiZ) variants of AAT was done. Genotyping is more appropriate for systematic analysis than quantitative measurement of serum levels, because alpha-1 antitrypsin deficiency is an acute phase reactant and serum concentrations may be increased in the course of any inflammatory process. In 65% of patients (54/83) simultaneously with genetic testing, alpha-1 antitrypsin deficiency serum level was measured.

Tab. 1. Possible causes of pancreatitis in examined patients*

No. of patients		Causes										
			Gene mutations									
		Anatomical anomalies	*PRSS1*	*CFTR*	*SPINK1*	*CTRC*	AAT	Hypertriglyceridemia/ hypercholesterolemia	Biliary tract disease	Abdominal trauma	Autoimmune pancreatitis	Positive family history
CP	65	11	10	5	9	4		2	2	3	1	16
ARP	4				1			2				1
AP	14				1		1		5	2		2

*Some of patients have more than one etiological factor

In view of the results as shown below it was not necessary to use statistical analysis.

RESULTS

Only in 1 of 83 patients with CP, ARP and AP alpha-1 antitrypsin deficiency was recognized. It was almost 13-years old, obese girl with CP, in which E342K mutation in one allele (PiZ heterozygote) and decreased alpha-1 antitrypsin deficiency serum concentration were found. The first and only episode of severe AP occurred at the age of 12.6 years. The performed imaging studies (ultrasound, CT), beyond enlargement of the organ and heterogeneously hypoechoic parenchyma, revealed multiple pancreatic cysts. During ERCP biliary stones were revealed, there were no signs of CP. Due to the lack of capacity to perform endoscopic cystogastrostomy and the ineffectiveness of conservative treatment the girl was qualified for surgery – subtotal resection of the pancreas with Roux-y loop and cholecystectomy. Histopathological examination shown increased fibrosis, resorption, with foci of necrosis and non-specific inflammatory infiltration with abscess formation. After surgery there were no further episodes of the AP, the girl requires pancreatic enzymes supplementation.

During over a dozen-week hospitalization because of AP also elevated levels of serum transaminases and hepatic steatosis in CT imaging were found. Therefore during pancreatic resection, at the same time, a liver biopsy was done. Histopathological examination shown massive mixed-nature steatosis covering 90% of hepatocytes, polymorphic inflammatory infiltration and foci of fibrosis in portal tracts.

DISCUSSION

Alpha-1 antitrypsin deficiency is a genetically determined disease, associated with the development of lung and/or liver injury. In majority of patients with AAT deficiency the disease is asymptomatic or oligosymptomatic. Hepatic form of the disease usually manifests itself in 4-8 weeks of age in the form of prolonged, cholestatic jaundice or neonatal hepatitis. It is also the second, after biliary atresia, most common cause of liver transplantation in children. In adults, the clinical manifestation of the disease is mainly related to the respiratory system (chronic obstructive pulmonary disease). AAT is one of the most important serum inhibitors of proteolytic enzymes such as trypsin, chymotrypsin, pancreatic elastase, leukocyte proteases and bacterial proteases. There is a hypothesis that increased levels of pancreatic proteinases or a decrease in pancreatic antiproteinases can lead to pancreatitis.

In our study only 1 of 83 patients with CP, ARP and AP was heterozygous for Z allele (E342K). There were no heterozygous patient for S allele (E264V) and no homozygous at all.

The reported incidence of AAT Z allele in polish population is 10.5 per 1,000 people (31). A single case of our patient is therefore only an accidental discovery compatible with the incidence of heterozygous allele Z in our population.

The speculations about AAT potential involvement in both chronic and acute pancreatitis were initiated by several studies (23-25, 32). In a study by Novis et al. 110 adult patients with CP (98 patients with alcohol-induced and 12 patients with idiopathic CP) were screened for the various AAT phenotypes. The results were compared with a control group of 116 blood-donors. The PiMZ phenotype was significantly more prevalent in the patients with CP than in control group (9 vs. 0%, $P < 0.001$). Serum concentrations of AAP were also more often below the reference range in the patients group than in controls (12 vs. 2%) (24).

Mihas and Hirshowitz measured alpha-1 antitrypsin deficiency serum level in a group of 15 patients with alcohol-related CP – mean AAP concentration was significantly lower in comparison with control subjects ($P < 0.001$) (33). Teich et al. detected moderating role of AAT variants in the course of chronic non-alcoholic pancreatitis (34). Other authors did not found an association between alpha-1 antitrypsin deficiency and pancreatitis. Lankisch et al. found no difference between serum alpha-1 antitrypsin levels in 76 patients with acute pancreatitis or CP compared to control group (28). Braxel et al. performed AAT phenotyping in 31 patients with AP and 59 patients with CP and detected no association between AAT phenotypes and pancreatitis (26). Similar results were obtained by Haber et al. comparing AAT phenotypes in 90 alcoholics with pancreatitis with 188 alcoholics without pancreatitis (29). In a largest so far published study performed by Witt et al. involving 96 unrelated children and adolescents with idiopathic or hereditary CP and 185 healthy controls, genotyping of

the S allele and the Z allele was done. Authors found no significant difference between the allele frequency in patients and the control individuals (P > 0.1) (27).

In the present study, genetic analysis of the two most common alpha-1 antitrypsin deficiency alleles PiS and PiZ revealed no association between AAT genotypes and the development of CP, ARP or AP.

CONCLUSIONS

In conclusion, it seems that a1-antitrypsin is not involved in the pathogenesis of pancreatitis in children. The lack of correlation between alpha-1 antitrypsin deficiency and pancreatitis in our study is in agreement with the results of most previous studies.

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