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Original article

The clinical course of hereditary pancreatitis in children – A comprehensive analysis of 41 cases

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ABSTRACT

Background: Available data from adult patients do not reflect natural course of hereditary pancreatitis (HP) in children. To date, no study has assessed the clinical course of HP in children.
Objective: To investigate the clinical course of HP in children and compare it to non-HP group with chronic pancreatitis (CP).
Methods: A group of 265 children with CP, hospitalized from 1988 to 2014, were enrolled in the study. Medical records of those patients were reviewed for data on presentation, diagnostic findings and treatment. All children were screened for mutations in major pancreatitis-associated genes, i.e. *PRSS1*, *SPINK1*, and *CFTR*.
Results: HP was diagnosed in 41 children (15.5%). Family history was positive in 88% of children with HP. Mutations of *PRSS1* gene were found in 80% (33/41) of HP patients. We detected p.R122H, p.R122C, p.N29I, and p.E79K mutation in 34% (14/41), 27% (11/41), 12% (5/41), and 7% (3/41) of HP patients, respectively. Patients with paternal inheritance had first symptoms earlier than those with maternal inheritance (5.9 vs. 9.1 years; $P < 0.05$). Children with HP showed more severe changes in ERCP then those from non-HP group (2.05 Cambridge grade, vs. 1.6°; $P < 0.05$). ESWL was performed more frequently in HP group (12.2% vs. 3.1%; $P < 0.05$). There was no difference in age of disease onset (7.98 vs. 8.9 years; NS), pancreatic duct stenting (46.3% vs. 33%; NS), or number of surgical interventions (12.2% vs. 14.3%; NS) between both groups.
Conclusions: Children with HP reveal significantly more severe clinical presentation of the disease than non-HP patients, despite the same age of onset.

Introduction

Chronic pancreatitis (CP), characterized by inflammation-induced continuous damage to the structure and function, or both, of the pancreas, is of rare occurrence in childhood. The etiology of CP in children varies and includes gene mutations, anatomic anomalies, metabolic disorders, and others [1–6]. Hereditary pancreatitis (HP) is a rare cause of CP, first described by Comfort in 1952 [7]. HP is an autosomal dominant disorder with penetrance of approximately 80–90% [8,9]. In 1996, association between HP and mutation (p.R122H) in the cationic trypsinogen gene (*PRSS1*) was described by Whitcomb et al. [10], which was further confirmed by an independent study [11]. Those publications started an intensive period of genetic studies in HP and CP which resulted in the discovery of over 25 new mutations in the *PRSS1* gene [12] and mutations in new genes associated with pancreatitis: *SPINK1* (serum protease inhibitor Kazal type 1) [13], *CFTR* (cystic fibrosis transmembrane conductance regulator) [14], *CTRC* (Chymotrypsin *C*) [15] and *CPA1* (carboxypeptidase A1) [16]. The

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diagnosis of HP is based on the presence of cationic trypsinogen gene mutation. In patients without *PRSS1* gene mutation, the diagnosis of HP is made when the patients satisfied the requirements of the family history (recurrent acute pancreatitis (RAP) or CP occurring in two first-degree relatives or three or more second-degree relatives, in two or more generations in the absence of precipitating factors) [8–10,17].

HP develops mainly in childhood [8–10,17,18]. The precise prevalence of HP in the general population is still unknown. In a French cohort from the national registry, the HP prevalence was estimated at 0.3/100 000 [8]. Recently, the prevalence of HP in Denmark in a large population-based cohort was estimated at 0.57/100 000 for HP patients and at 0.13/100 000 for carriers of *PRSS1* mutation [9]. The prevalence of HP patients in the CP group varies from 1% to 8% [18,20,21]. Available data from adult patients do not reflect the natural history of HP in children [8,9,17–20]. While adult patients smoke, drink alcohol and frequently have cardiovascular, respiratory and other comorbidities, these conditions are rarely observed in children. Another advantage of the analysis of pediatric population is the possibility to clearly evaluate such data as the age of the disease onset and number of exacerbations. Although the incidence of HP in the pediatric population is low, pancreatitis might cause significant morbidity and even mortality [1]. To date, no study has assessed the clinical course of HP in children. Most of the information is found within individual case reports or small case studies [22–27]. The recommendations on diagnosis and treatment in children with HP are still empirical, because prospective and retrospective trials in this group of children are lacking.

The Children's Memorial Health Institute in Warsaw, a leading national pancreatic center, admits the majority of Polish children with CP. Our group of children with CP (over 260 patients) is one of the largest single-center cohorts in the world. Thus, investigations of clinical presentation and treatment in this group could deliver essential findings for medical practice.

The aim of this retrospective study was to investigate the natural course of HP in children from a well-defined homogenous single-centre cohort.

Methods

Total of 265 children with CP hospitalized at the Department of Gastroenterology, The Children's Memorial Health Institute, Warsaw, Poland, between 1988 and 2014 were enrolled in the study. The inclusion criteria were: age $\leq$18 years, diagnosis of CP verified by imaging methods (US scan, CT, magnetic resonance cholangiopancreatography [MRCP], and/or endoscopic retrograde cholangiopancreatography [ERCP]), and follow-up of $\geq$12 months from time of the first visit. Children with acute pancreatitis, in whom CP was not confirmed according to the Cambridge classification system, were excluded from the study.

Moreover, all the participants were screened for mutations in the high-risk genes associated with CP. Written consents from patients and their parents were obtained before the analysis. The DNA was amplified by polymerase chain reaction (PCR) and sequenced using Sanger or RFLP (Restriction Fragment Length Polymorphism) method.

RFLP method and the direct sequencing of *PRSS1*, *SPINK1* and *CFTR* was performed in 21 and 20 cases of HP patients, respectively. Thirty out of 41 HP patients were analyzed for *CTRC* variants using direct sequencing. In HP cases (analyzed by direct sequencing), first exones 2 and 3 of *PRSS1* were analyzed. In the cases with no mutations detected (n = 8), the analysis of remaining exones (1,4,5) of *PRSS1* was performed.

Direct sequencing of all exones (1–5) of *PRSS1* was performed in 51 out of 224 non-HP patients. These 51 cases consisted of 40 idiopathic CP and 11 CP patients with various etiological factors. Since no mutations were detected, for the remaining non-HP patients the analysis was restricted to exone 2 and 3 which contain all pathogenic mutations of *PRSS1* found in Caucasian population (according to "Pancreas genetics" database; www.pancreasgenetics.org).

For *SPINK1* (exon 3) and *CTRC* (exon 2,3 and 7), the analyzed regions contained the most frequent or all known pathogenic mutations in *SPINK1* and *CTRC* gene, respectively (Pancreas genetics" database; www.pancreasgenetics.org). In *CFTR*, the most common mutations as found in Polish CP patients [3] were analyzed (exon 10, dele2,3(21 kb) or exon 9–11 and dele2,3(21 kb)). The fluorochromatograms were analyzed using Mutation Surveyor (Softgenetics, PA, USA) software with NM_002769.2 (*PRSS1*), NM_003122 (*SPINK1*), NM_000492.3 (*CFTR*), NM_007272.2 (*CTRC*) as reference sequences. Identified variants were named according to HGVS (Human Genome Variation Society) recommendations (http://www.hgvs.org). Molecular analysis was performed at the Department of Medical Genetics of Institute of Mother and Child, Warsaw, Poland (between 1988 and 2010, and 2012–14), Genomed S.A., Warsaw, Poland (2011–2012), or Medgen, Warsaw, Poland (2012).

In patients without *PRSS1* gene mutation, the diagnosis of HP was made when the patients satisfied the requirements of the family history. Pedigree was drown and relevant diagnoses, including pancreatic cancer (PC), were confirmed.

All patients underwent preceding radiographic studies, including abdominal ultrasound, CT, MRCP and/or ERCP. Endoscopic ultrasound (EUS) was not performed in these patients. Clinical data were recorded and analyzed. Family history, laboratory and genetic results, the results of imaging studies, surgical and endoscopic procedures were documented. All patients were monitored from their first pancreatitis attack. The first episode of acute pancreatitis, diagnosed on the basis of serum amylase activity $\geq$3 times over the upper normal range (reference value: 0–82 U/L), elevated urine amylase activity (reference value: 0–380 U/L), and serum lipase activity $\geq$5 times over the upper normal range (0–210 U/L), was regarded as the onset of CP. Disease activity was established based on the following parameters: age at onset, number of pancreatitis episodes, changes found on imaging (US scan and/or MRCP), changes in ERCP according to the Cambridge scale, results of the endocrine (fasting glucose, HbA_{1c}—all patients; if testing suggests an impaired glucose tolerance, oral glucose tolerance test was performed) and exocrine pancreatic function tests (all patients; at least one of the tests-the 72-hour fecal fat quantification, elastase-1 stool test, or breath test with ^{13}C-mixed triglycerides), nutrition status (BMI, Cole's Index), endoscopic and surgical procedures.

All patients were divided into 2 groups, depending on the etiological factor: Group 1 – patients with HP (*PRSS1* gene mutations or positive family history; n = 41), Group 2 – non-HP patients (children with other CP-causing etiological factors or idiopathic CP; n = 224).

Body Mass Index (BMI) was calculated from the formula: $BMI = actual\ weight\ [kg]/(height\ [m])^2$. The calculated value of BMI was compared with the data of the centile chart. In children, especially younger ones, Cole's ratio, i.e. the quotient of the actual and the standard BMI, is used to evaluate the anthropometric index: *Cole's ratio = (BMI actual/BMI for the 50th centile) × 100 [%]*. Normal Cole's ratio ranges between 90% and 110% [26].

The protocol of the study was approved by the Local Ethics Committee (38/KBE/2012).

Data were reported as mean ± standard deviation, or as median and range for continuous variables, and as relative frequencies for categorical variables. The chi-square test was used to compare relative frequencies. Analysis of continuous variables was

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