

Autoimmune markers in children with chronic pancreatitis

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Abstract

Introduction: In the last decade we can observe a gradual increase in the incidence of autoimmune diseases. The aetiology of chronic pancreatitis (CP) in children is varied and includes gene mutations, anatomic anomalies and others. The reported paediatric experience with chronic CP is scarce and little is known about the role of autoimmune pancreatitis (AIP).

Aim: To assess the frequency of autoimmune markers in children with CP.

Material and methods: One hundred and twenty-nine children hospitalised between 2005 and 2012 at the Department of Gastroenterology, The Children's Memorial Health Institute, were examined for the presence of AIP; the level of IgG4 was determined, and tests for anti-tissue antibodies (ANA, ASMA, AMA, ANCA, AHA) were conducted. Clinical data were recorded and analysed.

Results: Anti-tissue antibodies were detected in 75/129 children (58%), and 24/68 patients (35.3%) showed an increased IgG4 level. Based on the International Association of Pancreatology criteria, a suspicion of AIP was raised in 6 patients (4.6%). We found gene mutations predisposing to CP in 32/75 (42.6%) patients with autoimmune markers. In 16/75 children (21.3%), anatomic anomalies were found. There was no difference in the severity of the disease and clinical course between children with evidence of autoimmune process and patients without autoimmune markers ($p = NS$).

Conclusions: In children with CP, similarly to adults, there is a high frequency of biochemical markers of autoimmunity. It is worth remembering that AIP can occur in children.

Introduction

Autoimmune pancreatitis (AIP) was described for the first time by Sarles in 1961 [1]. It is a cause of acute pancreatitis, which can progress to the chronic condition in some cases. It is estimated that the latter constitutes 2–6% of all chronic pancreatitis (CP) cases in adult patients [2, 3]. Two types of the disease are included in the most recent classification. Type 1 occurs predominantly in adults, while type 2 is characteristic for younger patients, including children. It is frequently associated with inflammatory bowel diseases and responds better to glucocorticoid therapy. In some patients (up to 60%) AIP coexists with other autoimmune disorders, such as Sjögren's syndrome, systemic lupus erythematosus, diabetes or primary sclerosing cholangitis (PSC) [2–5].

The aetiology of CP in children is varied and includes gene mutations, anatomic anomalies, metabolic disorders and others [6–8]. In the last decade, a gradual increase in the incidence of autoimmune diseases in children can be observed. The pathogenesis of AIP is poorly understood and the frequency of autoimmune stigmata in children with CP is unknown. However, the literature on the subject is conflicting because most of the information about AIP in children originates from individual case reports or small case series [9–13]. Although the incidence of AIP in the paediatric population is low, pancreatitis may cause significant morbidity in children. Due to the infrequent occurrence of CP in childhood, clinicians may be unfamiliar with optimal diagnostic and management strategies of this condition.

The Children's Memorial Health Institute in Warsaw, a leading national pancreatic centre, admits the majority of Polish children with CP. Our group of children with CP (over 200 patients) is one of the largest single-centre groups in the world. Thus, investigations of autoimmune markers in this group could deliver essential findings for medical practice.

Aim

The aim of our study was to assess the frequency of autoimmune markers in children with chronic pancreatitis.

Material and methods

One hundred and twenty-nine children with CP, hospitalised between 2005 and 2012 at the Department of Gastroenterology, The Children's Memorial Health Institute (Warsaw, Poland), were enrolled into the study. The protocol of the study was approved by the Local Ethics Committee (121/KBE/2007).

The inclusion criteria were: age ≤ 18 years, diagnosis of CP verified by imaging methods (ultrasonography (US) scan, computed tomography (CT), magnetic resonance cholangiopancreatography (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP)), and follow-up ≥ 12 months. All patients had preceding radiographic studies, including abdominal ultrasound, CT and/or MRCP. Clinical data was recorded and analysed. Family history, laboratory findings, results of imaging studies and surgical and endoscopic procedures were documented. The first episode of acute pancreatitis, diagnosed on the basis of serum amylase activity ≥ 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 ≥ 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 disease onset, number of pancreatitis episodes, changes found on imaging (US scan and/or MRCP), changes in ERCP graded according to the Cambridge scale, results of the endocrine and exocrine pancreatic function tests, nutrition status (body mass index (BMI), Cole's Index) and endoscopic and surgical procedures.

All 129 children were examined for the presence of AIP; the level of IgG4 was determined, and the tests for anti-tissue antibodies: anti-nuclear antibodies (ANA), anti-smooth-muscle antibodies (ASMA), anti-mitochondrial antibodies (AMA), anti-liver/kidney microsome antibodies (LKM), anti-heart antibodies (AHA) and anti-neutrophil cytoplasmic antibodies (ANCA, both p-ANCA and c-ANCA), were conducted. The autoantibodies were determined in serum using indirect immunoflu-

orescence assay with specific substrates commercially supplied (EUROIMMUN): rat stomach (ASMA), rat liver and kidney (AMA, LKM), rat heart (AHA), HEP-2 cells (ANA) and human granulocytes (ANCA). All the tests were performed at the Department of Pathology of our institution.

The AIP was diagnosed according to the International Association of Pancreatology (IAP) guidelines, i.e. on the basis of immunological criteria (presence of antibodies: IgG4 and autoantibodies), radiological criteria (swelling of the pancreatic head and changes in the pancreatic duct) and response to corticosteroid therapy (Table I) [5]. However, we did not consider the morphological criterion (inflammatory infiltration of biopsy specimen) since none of our patients had the pancreatic biopsy.

Moreover, all the participants were screened for gene mutations predisposing to CP: *CFTR* (cystic fibrosis transmembrane conductance regulator; OMIM 602421), *PRSS1* (cationic trypsinogen/serine protease 1; OMIM 276000), and *SPINK1* (serum protease inhibitor Kazal type 1; OMIM 167790).

Statistical analysis

The results were subjected to statistical analysis. The χ^2 test was used to compare relative frequencies. Analysis of continuous variables was performed using the *U* Mann-Whitney and Kruskal-Wallis tests (Statistica for Windows, v5.0). Significance was assumed at $p < 0.05$.

Results

The presence of anti-tissue antibodies, suggesting the autoimmune character of pancreatitis, was detected in 75 out of 129 (58%) examined patients. The ANA at dilutions $\geq 1/80^e$ were present in 33 (25.8%) children with CP (including 6 at $\geq 1/320^e$, 5 at $\geq 1/640^e$ and one patient at $1/1280^e$).

Fifty (39.4%) children with CP had ASMA at dilutions $\geq 1/80^e$ (among them 22 at $\geq 1/160^e$, 4 at $\geq 1/320^e$ and

Table I. Specific features of autoimmune pancreatitis [2, 5]

Diffuse enlargement of the pancreas and changes in the pancreatic duct
Increased serum levels γ -globulins, IgG or IgG4
Presence of serum autoantibodies
Lymphocytic infiltration with fibrotic changes of biopsy specimen
Response to corticosteroid therapy
Co-existing autoimmune conditions

1 patient at 1/640^e). The AMA, LKM, AHA and ANCA were not detected in any of the examined sera.

Mutations of genes predisposing to pancreatitis were detected in 32/75 (42.6%) patients in whom the presence of anti-tissue antibodies suggested the autoimmune character of the condition. The list of affected genes included *PRSS1* ($n = 4$), *CFTR* ($n = 6$), *SPINK1* ($n = 16$) and both *CFTR* and *SPINK1* ($n = 4$). Furthermore, anatomic anomalies of the pancreatic duct, such as pancreas divisum ($n = 11$), ansa pancreatica ($n = 3$), and anomalous pancreaticobiliary union (ABPU, $n = 2$) were documented in 16/75 (21.3%) children from this group.

Clinically significant increase in IgG4 concentration was revealed in 24 out of 68 (35.3%) children who were examined for this parameter. The presence of mutations predisposing to pancreatitis (*PRSS1*, *CFTR*, *SPINK1*) was documented in 17/24 (71%) patients with elevated IgG4, and anatomical anomalies of the pancreatic duct were revealed in 5/24 (20.8%) individuals from this group.

Based on IAP criteria, a suspicion of AIP was raised in 6 patients (5 boys and 1 girl; aged 11–17 years). This diagnosis was definitely confirmed in 2 cases, based on clinical improvement observed after corticosteroid therapy. Due to the inactive phase of the disease, the immunosuppressive therapy was not implemented in the remaining suspected patients. Thirteen (6.3%) patients had coexisting chronic systemic autoimmune condition: colitis ulcerosa ($n = 6$), PSC ($n = 3$), Crohn's disease, dermatomyositis, panniculitis and juvenile arthritis ($n = 1$ each).

Gene mutations and anatomical anomalies of the pancreatic duct were the most common aetiological factors of CP in the studied group. The ERCP showed mean 1.68 grade according to the Cambridge Classification System.

There was no significant difference in the severity of the disease and clinical course between children with autoimmune stigmata and patients without autoimmune markers.

Discussion

In recent years, we have observed an increase in the number of children with CP in our hospital as well as in the other paediatric centres in the world. Unlike adult patients, in whom alcohol is the most common cause of CP, the aetiology of this condition in children is diverse [3, 6–8]. The majority of cases seen in our department resulted from gene mutations, anatomic anomalies of the pancreatic duct, biliary tract diseases and lipid disturbances [8, 14]. Due to improvements and wider availability of diagnostic modalities, not only is CP

detected with increasing frequency but also at earlier stages. At present, the diagnosis of CP in a younger patient is not as unique as it was several years ago [6, 7, 15]. However, analysis of the effects exerted by various aetiological factors on the outcome of this condition is hindered by the lack of a sufficiently large, homogeneous group of patients from a single centre. Longitudinal observation is vitally important. Available data from adult patients, mostly affected with alcoholic or biliary CP, does not reflect the clinical course of this condition in childhood [3]. The following questions have been raised: how various aetiological factors influence the severity of the condition in older age? Which of them constitute a true cause of the disease, and which only predispose to the pancreatitis? Analysing clinical data from children with CP, one can exclude the involvement of such factors as alcohol or cigarette smoking.

The AIP is estimated to constitute 2–6% of all cases of chronic pancreatitis in adults [2, 3]. In contrast, the data on the prevalence of this condition in children is lacking. Until recently, it was postulated that autoimmune chronic pancreatitis does not occur in paediatric patients. For many years, AIP was associated with the Far East, especially with Japan, as the majority of documented cases of this condition originated from this country [2, 3, 16]. A total of 16 children with autoimmune pancreatitis have been reported in literature thus far [11].

Usually, AIP is characterised by sudden onset. Pancreatitis is frequently accompanied by jaundice, general weakness and loss of weight. Focal changes of pancreatic parenchyma are often documented on diagnostic imaging. In view of the clinical manifestation and changes documented on imaging, pancreatic cancer followed by inflammatory masses associated with CP should be considered on differential diagnosis of AIP [2, 4, 9, 12, 17, 18]. Diagnostic imaging revealed pancreatic head masses in three of our patients with AIP. The AIP can also be asymptomatic, with the only abnormalities documented on laboratory tests and diagnostic imaging [2, 4, 9, 17].

The diagnosis is based on the diagnostic criteria of AIP developed by various societies and working groups. The most recent criteria of IAP were released in 2010 [5]. The list of AIP-characteristic parameters is presented in Table I.

On the basis of the IAP criteria, a suspicion of autoimmune pancreatitis was raised in 6 (2.9%) of our patients.

The high percentage of patients who showed biochemical markers of autoimmune process should be considered an interesting and somehow surprising finding of our study. The occurrence of autoantibodies

(non-specific ANA and ASMA) suggesting the autoimmune background of pancreatitis were detected in as many as in 75 out of 129 (58%) patients who were examined for the presence of autoimmune process. Clinically significant increase in the concentration of IgG4 was documented in 35.3% (24/68) of children tested for this parameter. These findings are consistent with the results reported by Uzan *et al.*, who documented the clinical and biochemical markers of autoimmune process in nearly 40% of adult patients with CP [19]. Furthermore, the present study confirmed our previous findings from a group of 41 children with CP. The presence of autoantibodies was documented in 17/41 (41.5%) patients from this group [10]. This evidence reflects our limited knowledge on the pathomechanism of CP. In our opinion, the high percentage of patients with CP showing biochemical markers of autoimmune process is associated with the chronic inflammation of pancreatic parenchyma. Chronic inflammation is associated with the release of autoantigens from pancreatic parenchymal cells. This is reflected by the synthesis of autoantibodies, as in the case of other chronic inflammatory conditions. The presence of biochemical markers of autoimmune process in paediatric patients with CP is a result of the disease rather than its cause. A considerable fraction of our patients with immunological markers of autoaggression showed other aetiological factors of CP, such as gene mutations and anatomical anomalies of the pancreatic duct. While the role of pancreas divisum or mutation in the *SPINK1* gene raises many doubts as to the potential direct causes of CP, mutations of *PRSS1* are unambiguously the direct reason for this condition [3, 8, 20]. Plausibly, those above-mentioned and other factors initiate the inflammatory process, while the immune markers of autoaggression appear at later stages. Therefore, positive results of tests for anti-tissue antibodies, even at low dilutions, and elevated serum concentration of IgG4 should be taken in one's stride. Furthermore, the lack of differences in the manifestation of CP in paediatric patients with and without markers of autoaggression may also point to their low clinical importance.

Undoubtedly, all other criteria of IAP, including pancreatic biopsy, should be satisfied in order to diagnose AIP in paediatric patients. The role of the histopathological examination in confirming the diagnosis of AIP was emphasized by Zen *et al.*, who reviewed current evidence regarding this condition [11]. Additionally, the authors of a recently published paper from the Mayo clinic recommend inclusion of EUS-guided pancreatic biopsy in the evaluation of AIP in paediatric patients [13]. Not only is the histopathological examination helpful in

confirming the diagnosis of AIP, but it can also distinguish between the types of this condition.

The most important limitation of our study is that it was a retrospective analysis based on medical history. Observational studies are less compelling than clinical trials. However, studies with large numbers of children may be difficult to conduct as CP is a very rare disease in children.

Conclusions

We would like to emphasise that although AIP is a rare cause of CP in children, it should be considered in differential diagnosis of this condition. One should remember the high prevalence of the immunological markers of autoaggression in paediatric patients with CP. Diagnosis of AIP in paediatric patients should be based on all criteria of IAP, including pathomorphological examination. Aside from diagnostic imaging and response to corticoid therapy, pancreatic biopsy is also required to establish the correct diagnosis of autoimmune pancreatitis in children.

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