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Alimentary Tract

Elevated serum antiphospholipid antibodies in adults with celiac disease

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ABSTRACT

Background and aims: An increased incidence of thrombosis is suggested in celiac disease. We explored serum levels of antiphospholipid antibodies in untreated and treated adult celiac disease patients.
Methods: A cohort of 179 biopsy-proven celiac disease patients (89 untreated, 90 on long-term gluten-free diet) and 91 non-celiac controls underwent clinical examination, assessment of celiac serology and enzyme immunoassay testing for anticardiolipin IgG and IgM, prothrombin IgG, and phosphatidylserine-prothrombin IgG and IgM.
Results: The level of antiphospholipid antibodies was higher in celiac disease patients compared with controls: anticardiolipin IgG 4.9 (0.7–33.8) vs 2.2 (0.4–9.6) U/ml, antiprothrombin IgG 2.9 (0.3–87.8) vs 2.1 (0.5–187.0) U/ml, antiphosphatidylserine-prothrombin IgG 6.9 (0.0–54.1) vs 2.3 (0.5–15.1) U/ml; $p < 0.05$ for all. Anticardiolipin IgG, antiprothrombin IgG and antiphosphatidylserine-prothrombin IgG were higher in treated compared with untreated patients. The phenotype of celiac disease at presentation (gastrointestinal symptoms, malabsorption or anemia, and extraintestinal symptoms or screen-detected disease) had no effect on the level of serum antiphospholipid antibodies.
Conclusion: The serum level of antiphospholipid antibodies is increased in adults with celiac disease. The higher level of antibodies in treated patients suggests that the increase is not gluten-dependent. The prothrombotic role of antiphospholipid antibodies in celiac disease warrants further studies.

1. Introduction

Celiac disease is characterized by dietary gluten-triggered inflammation and morphological damage to the small-intestinal mucosa. The classical gastrointestinal symptoms include abdominal pain and diarrhea, but patients also present with extraintestinal complaints such as dermatitis herpetiformis, arthralgias, infertility, neurologic problems and depression, or laboratory findings indicating nutritional deficiencies [1]. Celiac disease is an autoimmune-based condition, and autoimmune thyreoiditis and diabetes mellitus type 1 commonly occur together with it. The treatment of celiac disease is a life-long gluten-free diet which leads to a beneficial clinical, histological and immunological response in the vast majority of patients [1,2].

In inflammatory bowel diseases, e.g. Crohn's disease and ulcerative colitis, patients are at an increased risk of developing thrombosis especially during a disease flare. The mechanisms behind this prothrombotic tendency are very likely multifactorial and related to the inflammatory state [3,4]. Less is known about thrombotic complications in celiac disease, but an increased risk of thrombosis is noted in several case reports and a few observational studies [5]. In untreated celiac disease both hereditary and an abundance of acquired factors may predispose patients to thrombosis [6]. Even treatment of celiac disease might not solve the issue of thrombotic tendency, as there is evidence of persis-

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tent inflammation, i.e. small-bowel intraepithelial lymphocytosis, after long-term adherence to a gluten-free diet [7]. In inflammatory bowel diseases active prophylaxis and treatment of thromboembolic complications is considered appropriate despite the increased risk of gastrointestinal bleeding [3,4]. Regarding celiac disease, there are no recommendations or widely accepted consensus to guide clinical practice.

The autoimmune nature of celiac disease together with observations of thrombotic complications have prompted studies aiming to establish the prevalence of thrombophilic autoantibodies among patients with celiac disease. The most specific of these is that by Lerner et al. [6] on the celiac pediatric population, concluding that the incidence of antiphosphatidylserine-prothrombin (aPS/PT) IgG is increased in pediatric celiac disease patients. However, the activity of celiac disease in the study population was not addressed [6].

We now explored serum level of antiphospholipid antibodies cardiolipin IgG and M, prothrombin IgG, and aPS/PT IgG and IgM in adult celiac disease patients. Further, we sought to establish whether the level of antiphospholipid antibodies is different in untreated celiac disease patients from those on a long-time gluten-free diet. Finally, we investigated whether the serum antiphospholipid antibodies are associated with any particular clinical phenotype of celiac disease in the adult population.

2. Methods

2.1. Patients and study design

The study was conducted at the University of Tampere and Tampere University Hospital. The Ethics Committee of Tampere University Hospital approved the study protocol and all subjects gave written informed consent.

The celiac disease cohort consisted of 179 consecutive, prospectively studied patients. All diagnoses were confirmed with a small-bowel mucosal biopsy. Eighty-nine (50%) patients were previously undiagnosed and thus untreated. Three phenotypes of celiac disease at the time of diagnosis were recorded: gastrointestinal symptoms, malabsorption or anemia, and extraintestinal symptoms or screen-detected celiac disease. Ninety patients had been previously diagnosed and all had been adherent to a gluten-free diet for a median duration of 9 years (range 1–41 years). The non-celiac control group (n = 91) consisted of patients with abdominal complaints not due to celiac disease (n = 51) and healthy spouses of celiac disease patients (n = 40).

The study subjects (all ≥18 years old) underwent a thorough interview, clinical examination and gastrointestinal endoscopy with duodenal biopsy. Blood samples were drawn at the time of endoscopy and serum samples for the analysis of celiac disease serology phospholipid antibodies were frozen at −80 °C. Medical records were available for the collection of clinical data and routine laboratory measurements.

2.2. Measurement of antiphospholipid antibodies

Testing for the antiphospholipid antibodies cardiolipin IgG and IgM, prothrombin IgG, and aPS/PT IgG and IgM was carried out of thawed serum samples in SFS-EN ISO 15189: 2007 accredited laboratory at the Department of Laboratory Medicine, Seinäjoki Central Hospital, according to the manufacturer's protocol (AESKULISA, AESKU Diagnostics, Wendelsheim, Germany). The solid-phase enzyme immunoassay has previously been described in detail [6]. The positive cut-off for all antibodies was 18 U/ml.

2.3. Celiac serology and small-bowel mucosal histology

Serum IgA class transglutaminase 2 antibodies (TgA) were measured by an enzyme-linked immunosorbent assay (Celikey; Phadia, Freiburg, Germany) according to the manufacturer's instructions. Values ≥5.0 U/l were considered positive. Endomysial antibodies (EmA) were detected by a well-validated in-house method which uses human umbilical cord as a substrate and defines a titer 1:≥5 as positive [8].

Four to six well-oriented small-bowel mucosal biopsy samples were processed to examine villous height and crypt depth ratio (Vh/CrD) as previously described in detail [9]; a ratio <2.0 was considered abnormal. The density of CD3+ and γd+ intraepithelial lymphocytes (IELs) was analyzed from the frozen samples by immunohistochemistry as previously described [10]. The reference value was 37 cells/mm for CD3+ and 4.3 cells/mm for γδ IELs [11].

2.4. Statistics

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). Since all continuous variables were skewed, medians and ranges were calculated to describe the data. Numbers and percentages were used for categorical variables. Comparisons between the groups were based on Mann–Whitney U test or Kruskal–Wallis test in nonparametric variables and Chi-square test in categorical variables. Relationships between the continuous variables were examined using Spearman's rank correlation coefficient. The limit of significance was set at 0.05 (2-tailed).

3. Results

3.1. Demographic and clinical features

Demographic and clinical data on celiac disease patients and controls are set out in Table 1. Patients with celiac disease (n = 179) were older than the controls; median age 53 years, (range 18–86) and 45 years (range 19–84), respectively, p = 0.001. The distribution of gender as well as body mass index (BMI) was not different in celiac disease patients compared with controls. In the untreated celiac patient cohort, the occurrence of different disease phenotypes (gastrointestinal symptoms, malabsorption or anemia, and extraintestinal symptoms or screen-detected celiac disease) was equal.

Associated diseases in the 179 celiac disease patients included autoimmune thyroiditis or hypothyroidism in 21 (12%), rheumatoid disease in 7 (4%), diabetes mellitus type 1 in 4 (2%), bronchial asthma in 14 (8%), hypertension in 20 (11%), atherosclerosis in 12 (7%), and depression in 12 (7%) patients. Two treated celiac disease patients had experienced an episode of venous thrombosis, and one untreated patient had suffered recurrent miscarriages. Among the 91 controls, autoimmune thyreoiditis or hypothyroidism was noted in 6 (7%), rheumatoid disease in 4 (4%), diabetes mellitus type 1 in none, bronchial asthma in 1 (1%), hypertension in 2 (2%), atherosclerosis in 3 (3%), and depression in 2 (2%) patients.

3.2. Antiphospholipid antibodies in patients with celiac disease

Comparing all celiac disease patients (n = 179) with non-celiac controls (n = 91), higher levels of serum cardiolipin IgG, prothrombin IgG and aPS/PT IgG antibodies were observed in the former (Fig. 1). The levels of these antibodies were clearly associated with each other (for cardiolipin IgG and prothrombin IgG r = 0.585; for cardiolipin IgG and aPS/PT r = 0.845; and for prothrombin IgG and aPS/PT r = 0.627; p < 0.001 in all).

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