High Incidence of Celiac Disease in Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** To evaluate the presence of celiac disease in patients with systemic sclerosis (SSc). The association of autoimmune diseases with celiac disease has been reported, but few publications deal with the combination of SSc and celiac disease.

Methods. We investigated the presence of anti-tissue transglutaminase (anti-tTG) antibodies and serum antiendomysial antibodies (anti-EMA) in 50 patients with SSc. All subjects were on a gluten-containing diet. Duodenal mucosa histology and biopsy culture were performed in anti-tTG-positive patients; anti-EMA and IgA, IgG1 anti-tTG were detected in culture supernatants.

Results. The incidence of celiac disease in patients with SSc was found to be 8%. Serum anti-tTG antibody-positive results were detectable in 5 out of 50 patients with SSc, but only in 4 of them was the diagnosis confirmed by histological results (Marsh classification).

Conclusion. Our data show an increased prevalence of celiac disease in patients with SSc. (J Rheumatol First Release April 1 2009; doi:10.3899/jrheum.081000)

Key Indexing Terms:
CELIAC DISEASE AUTOANTIBODIES AUTOIMMUNE DISEASES SCLERODERMA

Systemic sclerosis (SSc) is a clinically heterogeneous disorder of the connective tissue characterized by immune activation, microvascular injury, and fibrosis. Major organ-based complications involving the lungs, heart, kidneys, and gastrointestinal (GI) tract determine mortality and morbidity. Clinical manifestations depend both on organ injury and on subsets of SSc: diffuse cutaneous SSc (dcSSc) is characterized by short-term Raynaud's phenomenon (RP), truncal and acral skin involvement, interstitial lung disease, renal failure, diffuse GI disease, myocardial involvement, erectile dysfunction; limited cutaneous SSc (lcSSc) is characterized by RP and limited skin involvement for many years, but also significant incidence of pulmonary hypertension, trigeminal neuralgia, skin calcification, and telangiectasia[1,2]. Irrespective of disease classification, SSc is typically associated with RP; up to 95% of patients with SSc have secondary RP. Scleroderma RP is characterized by microvascular damage and high plasma adrenomedullin and endothelin-l concentrations[3]. After the skin, the GI tract is the second most common target of SSc[4]. Digestive system involvement occurs in 50% of patients with SSc. Dyspepsia, restricted distension of the gastric antrum, and diffuse GI dysmotility are frequent features in SSc. These defects are independent from the occurrence of autonomic neuropathy[5]. Small bowel disease can be associated with a variety of symptoms such as intermittent bloating, abdominal cramps, chronic diarrhea, and malabsorption frequently requiring prokinetic drugs, laxatives, or proton-pump inhibitors, but the absence of symptoms occurs in about one-third of patients[6-8].

Celiac disease (CD), an autoimmune enteropathy induced by wheat gliadin in genetically susceptible individuals, is one of the most common immunologically mediated GI diseases in Europe[9]. This illness shows a multitude of symptoms and signs involving several organs, although the diagnosis is based on histological observation in the intestinal mucosa of gluten-dependent villous atrophy[10]. In its common form, the presence of autoantibodies against endomysium and tissue transglutaminase (tTG) antigens, together with their disappearance with a gluten-free diet, confirms the diagnosis[11,12]. Antiendomysial antibodies (EMA) and anti-tTG antibodies provide useful evidence for screening and followup of patients with CD[13-15]. Although definitive diagnosis of CD is still based on histological findings of villous atrophy in duodenal specimens, detection of EMA and anti-tTG antibodies may be useful if histological examination is not specific for CD[16]. CD may coexist with

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connective tissue diseases, but identification of this association is difficult because CD may be atypical or may mimic a rheumatologic condition; in addition, autoimmune diseases may show typical symptoms of CD. All these findings often create confusion and delay correct diagnosis[17]. Recent reports showed links between CD and other autoimmune disorders such as type I diabetes, autoimmune thyroiditis, Sjögren's syndrome, ankylosing spondylitis, polymyositis, rheumatoid arthritis, and psoriatic arthritis as well as autoimmune liver disease and Addison's disease[18-24].

Few publications have reported CD in patients with SSc[25-29]. The pathogenetic mechanism of the association between gluten enteropathy and other autoimmune diseases is still unclear: genetic predisposition, immunological mechanisms, and environmental factors have to be considered. Our aim was to investigate the incidence of CD in patients with SSc.

MATERIALS AND METHODS

Subjects. The study group consisted of 50 consecutive patients (43 female, 7 male; mean age 51 ± 14.5 yrs) with SSc diagnosed according to the American Rheumatism Association (American College of Rheumatology; ACR) criteria admitted to the Clinical Immunology and Allergy Unit of Sapienza University. All fulfilled the ACR criteria for SSc. They were subsequently divided into lcSSc and dcSSc subgroups according to LeRoy, *et al*[1]. Disease activity and disease severity in SSc was measured using the Valentini Scleroderma Disease Activity Score[30] and Medsger Scleroderma Disease Severity Scale[31]. Table 1 lists the main baseline epidemiological and clinical features of the patients.

All study procedures were in accord with the ethical standards of the institutional committee responsible for human experimentation.

Circulating antibodies. Serum was collected from all patients. IgA and IgG anti-tTG antibodies were detected using an ELISA in which microtiter plate wells were coated with recombinant human tTG (Eu-tTG kit; Eurospital, Trieste, Italy)[32,33]. According to the manufacturer's instructions, serum was diluted 1:100 and the absorbance was measured at 405 nm. The lower limit of positivity of IgG and IgA antibodies was, respectively, 29 AU/ml and 7 AU/ml. IgA and IgG1 EMA were screened by the direct immunofluorescent method on cryostat sections of monkey esophagus (Antiendomysium kit; Eurospital). IgA and IgG1 isotype EMA were measured in sera diluted 1:5 from all subjects showing anti-tTG-positive results[34]. EMA-positive results were identified by the typical reticulin-like staining of smooth-muscle bundles.

Duodenal biopsy examination. Four duodenal mucosa specimens were obtained at esophagogastroduodenoscopy. Two specimens were submitted for routine histological examination according to the Marsh/Oberhuber classification[35]. The results compatible with class III (a, b, and/or c) were considered pathognomonic for CD[35]. Two specimens were submitted to an organ culture system for 48 h at 37°C: one in the presence and one in the absence of a peptic-tryptic digest of gliadin (1 g/l) as described[36,37]. Culture supernatants were collected and stored at –70°C until they were used for both IgA and IgG1 EMA detection[36,37].

Statistical analysis. All results are expressed as mean ± standard deviation. Analysis was done for all the 50 patients. Differences in continuous variables were evaluated by Student t test. Chi-square test and Fisher's exact test were used for categorical variables. P value < 0.05 was considered significant.

Table 1. Epidemiological and clinical features of the study population (n = 50).

Feature	
Female/male	43/7
Age, mean ± SD yrs	51 ± 14.5
Age at onset of disease, mean ± SD yrs	40 ± 17
Raynaud's phenomenon duration, mean ± SD yrs	15 ± 10
Subset* dsSSc/lcSSc, n	27/23
ANA pattern, n (%)	
Speckled	9 (18)
Speckled and nucleolar	18 (36)
Nucleolar	4 (8)
Centromere	19 (38)
SSc-specific autoantibody, n (%)	
Anti-topo I	23 (46)
ACA	19 (38)
None	8 (16)
DAI**	1.8 ± 0.6
DSI†	4.7 ± 2.2
Disease features, n (%)	
Interstitial lung disease	23 (46)
Scleroderma renal crisis	3 (6)
Myositis	4 (8)
Raynaud's phenomenon	50 (100)
Gastroesophageal reflux disease	45 (90)
Digital ulcer	22 (44)
Pulmonary hypertension	18 (36)
Sicca syndrome	6 (12)
Autoimmune thyroiditis	8 (16)
Primary biliary cirrhosis	4 (8)
Previous or current iron deficiency anemia	7 (14)
IgA deficit	1 (2)
Positive family history for celiac disease, n (%)	1 (2)

dcSSc: diffuse cutaneous SSc: lcSSc: limited cutaneous SSc: ANA: antinuclear antibody; anti-topo I: anti-topoisomerase I, ACA: anticentromere antibody. * According to Le Roy, *et al*[1]; ** Disease Activity Index according to Valentini, *et al*[30]; †Disease Severity Index according to Medsger, *et al*[31].

RESULTS

Five of 50 (10%) SSc patient samples were positive for IgA anti-tTG antibodies in sera. Table 2 lists the main baseline epidemiological and clinical features of the patients presenting anti-tTG antibodies in sera. One of these 5 patients also was positive for IgG anti-tTG. IgA EMA-positive results were present in only 2 of 5 patients (Table 3). Four of 5 patients presenting IgA anti-tTG antibodies and/or EMA-positive results underwent endoscopic examination, and one refused this examination. These 4 patients showed subtotal or total atrophy of intestinal mucosa on histological examination (types IIIa to IIIc of the Marsh classification modified by Oberhuber, *et al*[35]). Organ culture investigations showed positive EMA and anti-tTG antibodies in 2 (50%) of the 4 SSc patients showing total atrophy of intestinal mucosa; specimens of the other 2 were excluded because of tissue necrosis (Table 3).

Esophagogastroduodenoscopy was performed for routine checks of gastroesophageal reflux disease in 20 patients with serum IgA anti-tTG antibody or EMA-negative results. In all patients, standard endoscopy showed no mucosal features (loss of duodenal folds, scalloping, nodularity, mosaic)

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Table 2. Epidemiological and clinical features of sera from 5 patients who were anti-tTG-positive.

Feature	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	p
Sex	F	F	M	M	M	NS
Age, yrs	20	68	34	46	69	NS
Age at disease onset, yrs	18	60	23	40	65	NS
RP duration, yrs	3	8	26	7	29	NS
Disease subset	dcSSc	dcSSc	dcSSc	dcSSc	lcSSc	NS
ANA pattern	Speckled	Speckled/Nuc	Speckled/Nuc	Speckled/Nuc	ACA	NS
SSc-specific autoantibody	None	Anti-topo I	Anti-topo I	Anti-topo I	ACA	NS
History of iron deficiency anemia	Yes	Yes	Yes	Yes	No	NS
Celiac disease symptoms	Yes	Yes	Yes	Yes	No	

RP: Raynaud's phenomenon; dsSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; ANA: antinuclear antibody; Nuc: nucleolar; anti-topo I: anti-topoisomerase I; ACA: anticentromere antibody; NS: nonsignificant.

Table 3. Histology, culture IgA EMA and anti-tTG antibodies, and IgA EMA-positive results in serum from 5 patients who were IgA anti-tTG-positive.

		Culture Supernatants		Serum
	Intestinal Atrophy*	IgA EMA	IgA Anti-tTG	IgA EMA
Serum IgA anti-tTG-positive patients	4/5	2/4	2/4	2/5

* Type III of the Marsh classification as modified[35]. EMA: anti-endomysial antibodies; tTG: tissue transglutaminase.

suspicious for CD and alerting the endoscopist to take small intestinal biopsy specimens. However, biopsy specimens were taken from the duodenal bulb in 6 patients. The histological examination in these patients was normal.

In the study population of SSc patients the prevalence of CD was 8% according to detection of serum anti-tTG antibodies and histological classification. Results from the 50 SSc patients showed no correlation between the presence of CD and epidemiological or clinical findings (sex, age, disease duration, onset of RP, SSc subset, antinuclear antibody pattern, and SSc-specific autoantibody).

DISCUSSION

The increased prevalence of CD in patients with autoimmune pathologies has been reported in several studies, although the role of CD in the clinical course of different autoimmune diseases is unclear.

We found increased IgA anti-tTG antibodies in serum samples from 5 (10%) patients with SSc, but only 2 of them presented IgA EMA-positive results. Histological findings, according to the Marsh classification[35], were positive for all of the 4 patients examined. Further, we found that EMA and anti-tTG antibodies were detectable in 2 culture supernatants of 4 patients studied; specimens of 2 of them were not adequate for evaluation because of tissue necrosis. Although screening for CD routinely evaluates the presence of specific serological markers (anti-tTG and EMA antibodies) in patients' sera, confirmation of CD is still based on the histological observation of villous atrophy in duodenal specimens. In the presence of CD symptomatology, culture supernatants may be useful if histological examination is negative or uncertain. EMA assay from a cultured intestinal biopsy can detect gluten-sensitive enteropathy, characterized by an infiltrative/hyperplastic histological pattern, that is often associated with negative serum EMA[38]. However, culture supernatants may give a negative result from specimens of small size or with tissue necrosis.

Our previous data suggested that if anti-tTG antibodies were present in EMA-negative patients it could be indicative only of tissue lesion and not expression of CD[39-41]. Indeed it is possible to hypothesize that tTG is not the only antigen of the EMA. One report described that although tTG immunoabsorption is enough to abrogate reactivity of the anti-tTG ELISA, it fails to completely rule out EMA binding activity[42]. Moreover, another study showed the existence of EMA antigenic epitopes not related to tTG[43]. Tissue transglutaminase, the autoantigen well recognized for its role in the pathogenesis of CD, could perhaps not be the only autoantigen involved in gluten-dependent autoimmune reactions. Other normally "cryptic" autoantigens could be unmasked and cause a self-aggressive immunological response following the gliadin-initiated inflammatory process[44]. Immune response to cryptic antigens is not the only pathogenetic mechanism of autoimmune disease: genetic predisposition and molecular mimicry (triggering antigens) may also play a role.

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In addition to genetic predisposition and exposure to triggering antigens, development of autoimmune diseases is related to the loss of mucosal barriers connected to environmental factors[45].

Finally, the duration of gluten exposure following the gliadin-initiated inflammatory process has been reported to be a cause of the high prevalence of autoimmune disorders in celiac patients[46]. It appears that organ-specific autoantibodies are gluten-dependent and disappear with a correct gluten-free diet[11].

In our study, CD was confirmed by the presence of villous atrophy in duodenal specimens in only 4 patients (8%). In SSc patients with GI symptoms and iron deficiency anemia, it is important to investigate for coexistence of CD. It is not known if the association of these 2 diseases is linked to the presence of a common pathogenic mechanism (an immune response against self-antigens) and/or a common genetic background.

Our study demonstrated a high prevalence of celiac disease among patients with SSc. Routine screening for celiac disease should be considered in patients with SSc.

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