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High prevalence of gluten sensitivity in a cohort of patients with undifferentiated connective tissue disease

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Key words

Undifferentiated connective tissue disease (UCTD); celiac disease (CD); gluten sensitivity; anti-nuclear antibody (ANA); hepatitis C virus (HCV) infection

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Summary

Objectives: *The aim of this study was to investigate if co-morbid conditions as hepatitis C virus infection and celiac disease may be associated to undifferentiated connective tissue disease.* ***Methods:*** *We studied retrospectively and prospectively 52 patients with diagnosis of undifferentiated connective tissue disease, subdivided, according to Vaz criteria, in systemic lupus erythematosus, systemic sclerosis and Sjögren's syndrome-like subgroups. Serological markers of celiac disease as anti-gliadin, anti-endomysium and anti-tissue transglutaminase antibodies were investigated. An esophagogastroduodenoscopy with duodenal biopsy and histological examination was proposed to patients with positive celiac disease serology. In addition antibodies directed to hepatitis C virus and total IgA-antibodies were investigated.* ***Results****: Six patients (11,5%) were positive for celiac disease serological tests although two of them were asymptomatic. Four patients underwent an esophagogastroduodenoscopy, showing total or subtotal villous atrophy at duodenal biopsies. Hepatitis C virus serology was negative in all patients and none had IgA deficiency. 83% of celiac patients showed a scleroderma-like phenotype. We observed a statistically higher incidence of autoimmune symptoms in patients with gluten sensitivity. Fatigue and myalgia regressed early after the beginning of gluten-free diet.* ***Conclusions:*** *In our cohort of patients the prevalence of celiac disease was higher than that reported in the general population. We believe that all patients with diagnosis of undifferentiated connective tissue disease, especially those with a systemic sclerosis-like presentation, should be investigated for celiac disease, even in absence of gastrointestinal symptoms. Gluten-free diet should be early recommended to all patients having undifferentiated connective tissue disease and gluten sensitivity.*

Introduction

Undifferentiated connective tissue disease (UCTD) is an autoimmune disorder with positive antinuclear antibody (ANA) results, at least one clinical manifestation of connective tissue disease (CTD) for at least three years but not fulfilling the classification criteria for any defined CTD (1). In about 70% of cases UCTD remains stable over years, and rarely shows spontaneous or treatment-induced regression or, on the other hand, progression to a defined CTD, especially SLE (2). Vaz et al divided UCTD patients into three subgroups according with signs and symptoms of patients: a systemic lupus erythematosus (SLE)-like subgroup, a systemic sclerosis (SSc)-like subgroup and a Sjögren's syndrome (SS)-like subgroup (3).

The aim of this study was to investigate co-morbid conditions that may induce or modify clinical and immunological manifestations of UCTD. We focused on celiac disease (CD) and chronic hepatitis C virus (HCV) infection, two conditions that

frequently pass unrecognized and that are known to be associated with some autoimmune diseases (4-11).
The prevalence of CD in unselected populations in North America and Western Europe falls within the range of 0.5%-1.26%, while in Italy it is between 0.2% and 0.94% (12).
Although a definitive diagnosis of CD is usually based on the histological finding of villous atrophy in duodenal biopsy, the currently available serologic tests for the diagnosis of CD include anti-gliadin (AGA), anti-endomysium (EMA) and anti-tissue transglutaminase (tTG) antibodies, (13,14).
We show here that, while HCV infection is not more frequent in UCTD, the prevalence of CD is increased in patients with UCTD and SSc-like symptoms compared to the general population.

Patients and Methods

This study involved 52 UCTD patients (1 male and 51 females aged 21 to 69 years, median 44 years) referred to the Department of Internal Medicine and Clinical Immunology at Sapienza University of Rome. Classification of UCTD was done according to the criteria proposed by Mosca et al (1). Patients were further classified in subgroups according to the criteria of Vaz et al (3). Patients were interrogated about having a previous diagnosis of CD; five of them were known to be celiac, and three of them were on gluten-free diet from few months to four years at the moment of the study. Three patients having UCTD and CD showed, at capillaroscopy, major abnormalities resembling scleroderma-like pattern (i.e. haemorrhages, dilated capillaries, and giant capillaries), in other two patients with UCTD and CD we observed damages of medium entity (i.e. elongated and tortuous capillaries, haemorrhages); only one celiac patient showed non-specific capillaroscopic changes (i.e. dilated and tortuous capillaries). Patients showed these abnormalities in spite of gluten-free diet. The remaining 47 patients were prospectively investigated for serological markers of CD, irrespective of the presence or absence of signs and symptoms suggesting CD; non celiac patients showed, at capillaroscopy, non-specific abnormalities. Patients with UCTD and CD showed chronic diarrhea, weight loss, abdominal distention, iron deficiency or anemia and recurrent abdominal pain associated with RP, ANA positivity and other rheumatic symptoms, whereas in patients with UCTD only we did not observe significant gastrointestinal symptoms but rheumatic disease symptoms only. The diagnosis of CD was, in almost all patients, preceding with respect to UCTD diagnosis, and we knew it studying retrospectively patients' clinic history; we diagnosed UCTD observing signs and symptoms resembling rheumatic disease only. Serum IgA levels were measured in all patients by nephelometry (Nephelometer BN Prospec, Siemens) to exclude IgA deficiency; HCV serology was investigated in 31 of the 52 patients by third generation enzyme linked immunosorbent assay (Roche Diagnostics, Mannheim, Germany). IgA and IgG1 EMA were tested by indirect immunofluorescence, and IgA and IgG anti-tTG antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) test, as described elsewhere (15,16). Conventional AGA were tested by a sandwich type enzyme immunoassay, used for the quantitative determination of IgA/IgG specific antibodies directed against the α-fraction of wheat gluten gliadin. The cut-off values, provided by the manufacturer, were 16.0 UA/ml and 50.0 U/ml for AGA IgA and IgG, respectively.
Esophagogastroduodenoscopy with duodenal biopsy was proposed to the patients with positive serological tests. In the course of esophagogastroduodenoscopy, three or four oriented duodenal biopsy were obtained, and histological examination was performed in accord to Marsh/Oberhuber classification (17). Results belonging to class III were considered compatible with CD diagnosis. A gluten free diet was recommended to all patients with a CD diagnosis. Unpaired data were analysed by the Mann-Whitney U-test, a P value of less than 0.05 was considered statistically significant.

Results

The most common clinical manifestations in 52 UCTD patients were arthralgia (58%), fatigue (35%), sicca syndrome (31%), Raynaud's phenomenon (RP) (25%) and myalgia (15%).
UCTD patients were sub-divided into 3 subgroups according to the criteria proposed by Vaz et al. (3): SLE-like, SSc-like and SS-like (**table 1**). 25 subjects (48%) were included in the SLE-like group, 14 (27%) in the SSc-like and 13 (25%) in the SS-like, on the basis, respectively, of the presence of fever, fatigue, arthralgia, myalgia for the SLE-like group; RP and capillary abnormalities on capillaroscopy for SSc-like group and sicca-syndrome for SS-like group. None of UCTD patients had IgA deficiency neither positive serology for HCV. Six of the patients (11.5%) were positive for serological markers of celiac disease. All six patients were positive for anti-tTG IgA and IgG, three of them were also positive for EMA IgA and IgG and only one was positive for AGA IgA and IgG. Four of these six subjects underwent esophagogastroduodenoscopy with multiple duodenal biopsies while two refused to perform this procedure. The histological evaluation of duodenal biopsies showed total or subtotal villous atrophy of bowel mucosa (IIIA type of the Marsh/Oberhuber classification). Only four subjects were symptomatic for celiac disease, presenting abdominal pain, diarrhea and iron deficiency anemia. In symptomatic celiac patients having UCTD almost all gastrointestinal symptoms regressed after the beginning of gluten-free diet whereas the majority of rheumatic disease signs and symptoms did not.
Five of the six subjects (83%) with gluten sensitivity belonged to SSc-like group (presence of RP and capillary abnormalities on capillaroscopy) and one to the SLE-like group (**table 2**). We also observed, in patients with UCTD and gluten sensitivity

compared to patients with only UCTD, a higher prevalence of autoimmune related symptoms such as arthralgia ($p < 0,027$), fatigue ($p < 0,008$), RP ($p < 0,0002$), myalgia ($p < 0,0002$) and thyroiditis ($p < 0,0076$). On the contrary, sicca syndrome had the same prevalence in the two groups ($p = 0.8$). Some of these autoimmune symptoms, particularly fatigue and myalgia, regressed after few months of gluten-free diet.

Discussion

Two of the six CD patients were asymptomatic for CD, and were characterized only by the autoimmune profile of UCTD. It is therefore important to consider a screening for CD in patients with a diagnosis of UCTD irrespective of intestinal symptoms or signs of malabsorption.

In the group of patients with CD, compared to the group with UCTD only, we observed a more severe clinical picture, characterized by an expanded spectrum of the typical symptoms of UCTD. In patients showing an SSc-like phenotype, anticentromere or anti-Scl70 antibodies were absent, suggesting that the induction of SSc-like manifestations by CD may be unrelated to that of specific autoantibodies. Because of the well-known association of HCV infection with autoimmune disorders (9), we investigated 31 of the 52 UCTD patients for HCV seropositivity, but we did not find a higher prevalence of HCV infection compared to the general population.

In conclusion, although in a small group of patients, we could observe a higher prevalence of CD in UCTD patients compared to general population. Therefore we suggest that serological screening for CD should be performed in all patients with UCTD, and especially in those with a scleroderma-like phenotype, even if without signs of malabsorption.

At present we don't know if a free gluten diet may contribute to regression or improvement of the clinical manifestations of UCTD, or may even prevent the clinical evolution to CTD. A longer follow-up study will give us this information and will further clarify the role of gluten sensitivity in autoimmune diseases.

References

1. Mosca M, Tani C, Vagnani S, Carli L, Bombardieri S. J Autoimmun. 2014;(48-49):50-2.

Table 1 *- Epidemiological and clinical features of 52 patients with UCTD diagnosis.*

UCTD pattern	N° (M/F)	Age (median, range)	Time from diagnosis (months; median range)	Previous IS therapy[1]	Diagnosis of CD[1]
SS-like	13 (0/13)	50 (68-30)	36 (1-60)	2 (15%)	0
SLE-like	25 (1/24)	40 (21-69)	36 (1-60)	6 (24%)	1 (4%)
SSc-like	14 (0/14)	39 (26-68)	12 (1-120)	6 (43%)	5 (36%)

IS: immunosuppressive

[1]number of patients (%)

Table 2 *- Clinical features of the 6 UCTD patients with gluten sensitivity.*

Case	UCTD pattern	Autoantibodies at diagnosis	CD-like symptoms	CD-associated antibodies	Intestinal biopsy	Previous IS therapy
1	SSc-like	ANA 1:160 speckled	Yes	EMA, tTG	Atrophy	Hydroxyurea
2	SSc-like	ANA 1:80 nucleolar	No	EMA, tTG, AGA	Atrophy	None
3	SSc-like	ANA 1:80 homogeneus	No	tTG	N.D.	Steroids
4	SSc-like	ANA 1:640 speckled	Yes	EMA, tTG	N.D.	None
5	SSc-like	ANA 1:640 speckled	Yes	tTG	Atrophy	Methotrexate, hydroxyurea, steroids, FANS
6	SLE-like	ANA 1:80 homogeneus	Yes	tTg	Atrophy	None

IS: immunosuppressive

2. Mosca M, Neri R, Bombardieri S. Undifferentiated connective tissue diseases (UCTD): a review of the literature and a proposal for preliminary classification criteria. Clin Exp Rheumatol. 1999;17(5):615-20.
3. Vaz CC, Couto M, Medeiros D. Miranda L, Costa J, Nero P, Barros R, Santos MJ, Sousa E, Barcelos A, Ines L. Undifferentiated connective tissue disease: a seven-center cross-sectional study of 184 patients. Clin Rheumatol. 2009;28(8):915-21.
4. Franzese A, Iafusco D, Spadaro R, cavaliere O, Prisco F, Auricchio R, Troncone R, Valerio G. Potential celiac disease in type 1 diabetes: A multicenter study. The Study-Group on Diabetes of Italian Society of Pediatric Endocrinology and Diabetology (ISPED). Diabetes Res Clin Pract. 2011;92(1):53-6.
5. Ch'ng CL, Jones MK, Kingham JG. Celiac disease and autoimmune thyroid disease. Clin Med Res. 2007;5(3):184-92.
6. Elfström P, Montgomery SM, Kämpe O, Ekbom A, Ludvigsson JF. Risk of primary adrenal insufficiency in patients with celiac disease. J Clin Endocrinol Metab. 2007;92(9):3595-8.
7. Luft LM, Barr SG, Martin LO, Chan EK, Fritzler MJ. Autoantibodies to tissue transglutaminase in Sjögren's syndrome and related rheumatic diseases. J Rheumatol. 2003;30(12):2613-9.
8. Rosato E, De Nitto D, Rossi C, Libanori V, Donato G, Di Tola M, Pisarri S, Salsano F, Picarelli G. High incidence of celiac disease in patients with systemic sclerosis. J Rheumatol. 2009;36(5):965-9.
9. Ramos-Casals M, Muñoz S, Medina F, Jara LJ, Rosas J, Calvo-Alen J, Brito-Zeròn P, Forms X, Sànchez-Tapias JM. Systemic autoimmune diseases in patients with hepatitis C virus infection: characterization of 1020 cases (The HISPAMEC Registry). J Rheumatol. 2009;36(7):1442-8.
10. Antonelli A, Ferri C, Fallahi P, Ferrari SM, Ghinoi A, Rotondi M, Ferrarini E. Thyroid disorders in chronic hepatitis C virus infection. Thyroid. 2006;16(6): 563-72.
11. Noto H, Raskin P. Hepatitis C infection and diabetes. J Diabetes Complications. 2006;20(2): 113-20.
12. Dubé C, Rostom A, Sy R, Cranney A, Saloojee N, garritty C, Sampson M, Zhang L, Yazdi F, Mamaladze V, Pan I, macneil J, Mack D, Patel D, Moher D. The prevalence of celiac disease in average-risk and at-risk Western European populations: a systematic review. Gastroenterology. 2005;128(Suppl 1):S57-67.
13. Farrell RJ, Kelly CP. Coeliac sprue. N Engl J Med. 2002;346(3):180-8.
14. Van der Windt DA, Jellema P, Mulder CJ, Kneepkens CM, van der Horst HE. Diagnostic testing for celiac disease among patients with abdominal symptoms: a systematic review. JAMA. 2010;303(17):1738-46.
15. Picarelli A, Sabbatella L, Di Tola M, Vetrano S, Casale C, Anania MC, Porowska B, Vergari M, Schiaffini R, Gargiulo P. Anti-endomysial antibody of IgG1 isotype detection strongly increases the prevalence of celiac disease in patients affected by type I diabetes mellitus. Clin Exp Immunol. 2005;142(1):111-5.
16. Fasano A, Catassi C. Clinical Practice. Celiac disease. N Engl J Med. 2012;367(25):2419-26.
17. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999;11(10):1185-94.