BRIEF COMMUNICATION

AG-2020-116
doi.org/10.1590/S0004-2803.202100000-39

Seronegative celiac disease in Brazilian patients: a series of cases

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Received: 23 June 2020
Accepted: 6 January 2021

ABSTRACT – **Background** – Celiac disease (CD) is an autoimmune disease characterized by immune reaction mostly to wheat gluten. The diagnosis is based on clinical, serological and histological findings in patients ingesting gluten. Cases that the clinical profile indicates CD and the autoantibodies are negative bring so a dilemma for the professional, as the risk of missed the diagnosis or a delay at the same. **Objective** – To show the importance of correct diagnosis of cases with seronegative celiac disease (SNCD). **Methods** – Ten cases of SNCD Brazilian patients were retrospectively studied (2013 to 2019). Data of clinical complaints, autoantibodies, IgA serum levels, histological findings and HLA-DQ2/DQ-8 were compiled. Dual-X densitometry, delay at diagnosis, previous autoimmune diseases and family history of CD were also checked. **Results** – All SNCD patients presented clinical symptoms of CD, with confirmed diagnosis by histological findings of the duodenal mucosa and HLA-DQ2 and/or HLA-DQ8 positivity. All patients had normal IgA levels and negative autoantibodies (IgA-anti-transglutaminase and anti-endomysial). Dual-X densitometry detected osteopenia in two women and osteoporosis in two males, all with low levels of vitamin D. Delay diagnostic ranged from 1 to 19 years. Familiar occurrence of CD was reported in 40% of the cases. After one year of gluten-free diet, eight patients refer improve of symptoms, while duodenal biopsies, done in five cases, showed histological improvement. **Conclusion** – Patients who demonstrate the clinical profile of celiac disease with negative serology and normal levels of IgA, especially those who have family members with celiac disease, should be submitted to duodenal biopsies to look for histological findings.

Keywords – Celiac disease; autoantibodies; diagnosis.

INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated disease triggered by the exposure to dietary gluten with strongly genetical influence, specially related to the presence of HLA DQ2/DQ8.[1] Usually the diagnosis is based on clinical symptoms, positivity of autoantibodies and histologic findings in patients ingesting gluten[1,2]. However, there are cases when the clinical profile indicates CD and the autoantibodies are negative, causing a dilemma for the professional[3]. CD diagnosis can be missed if the serology is negative[4]. The objective of this study is to present a series of cases with seronegative celiac disease (SNCD).

METHODS

This study was approved by the Ethics Committee of the Evangelical Beneficent Society of Curitiba (CAAE 84793318.0.0000.0103). This is a retrospective study performed through a survey of clinical charts. The same physician attended all the patients in a single private practice in the city of Curitiba, Brazil, during the period 2013 to 2019.

All included patients were diagnosed with CD based on clinical complaints, HLA-DQ2 and/or HLADQ-8 positivity and confirmation by histological findings of the duodenal mucosa, according to the Marsh classification[5]. The complaints registered were aphtha, gastroesophageal reflux, epigastric pain, indigestion, nausea, vomiting, flatulence, abdominal pain, diarrhea, constipation and abdominal distension, depression and anxiety. In addition, weighting, routine laboratory tests and Dual-X ray absormetry was done. Delay at diagnosis, family history of CD and previous autoimmune diseases was checked. Differential diagnosis with parasitic, infectious or inflammatory diseases, and the use of drugs, were ruled out as preconized by anamnesis and appropriate tests.

All the patients were simultaneously tested for serum levels of IgA and for autoantibodies class IgA-anti-tissue transglutaminase and IgA anti-endomysial. Upper endoscopy was performed with two or three biopsies of the duodenal bulb and at least four specimens post-bulbar[2]. Standard haematoxylin and eosin (H&E) was used for the analysis of the fragments. Experienced gastrointestinal pathologist referred results according to Marsh classification[5]. HLA-DQ2 and HLA-DQ8 presence were tested using DNA amplified by polymerase chain reaction.

After diagnosis, the patients were informed about CD and how to adhere to a gluten-free diet (GFD). After one year, they were clinically and laboratory reevaluated and a second duodenal biopsies were performed.

RESULTS

Were studied ten Caucasian Brazilian patients, seven women and three men, median age 30.5 years (range 16 to 68 years), who presented clinical symptoms suggestive of CD and negative autoantibodies (IgA-antiendomysial and IgA-anti tissue transglutaminase). Familiar occurrence of CD was reported in 40% of the cases (three mothers and one sister). The demographic, clinical and laboratorial findings were demonstrated in TABLE 1.

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
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TABLE 1. Demographic, clinical and laboratorial findings of patients with celiac disease seronegative.

Patient	Gender	Age	Delay diagnostic	Weight	Dual-X densimetry	Iron plasmatic	B12 plasmatic	Upper Endoscopy	HLA-DQ2	HLA-DQ8	Marsh Classification	Second biopsy* after one year
1	Female	18	NA	Normal	Osteopenia	Normal	Normal	Normal	Positive	Positive	II	NA
2	Female	19	5 years	Normal	Normal	Normal	Normal	Atrophy	Positive	Negative	III	NA
3	Female	19	11 years	Dim	Normal	Dim	Dim	Atrophy	Positive	Negative	III	I
4	Female	30	4 years	Over	Normal	Dim	Dim	Normal	Positive	Negative	II	NA
5	Female	31	NA	Normal	Normal	Dim	Dim	Normal	Negative	Positive	II	I
6	Female	39	1 year	Over	Normal	Normal	Normal	Atrophy	Positive	Negative	III	I
7	Female	57	1 year	Normal	Osteopenia	Dim	Dim	Normal	Positive	Negative	II	I
8	Male	16	11 years	Over	Osteoporosis	Normal	Normal	Nodular	Negative	Positive	III	NA
9	Male	32	19 years	Normal	NA	Normal	Normal	Duodenitis	Positive	Negative	II	NA
10	Male	68	NA	Normal	Osteoporosis	Normal	Normal	Duodenitis	Positive	Positive	I	I

NA: not available; Dim: diminished; GFD: gluten free diet. *According Marsh Classification. Note: all the patients had normal IgA levels for age.

In relation to symptoms, abdominal pain, abdominal distension, flatulence and gastroesophageal reflux were referred in 80%; depression in 60%; epigastric pain in 50%; diarrhea, constipation, aphtha and indigestion in 30%; nausea and vomit in 20%.

In nine cases a Dual-X densitometry was performed. Five patients had normal results. Osteopenia was observed in two cases (both women, with 18 and 57 years), while osteoporosis was detected in two males (16 and 68 years). All the patients had low levels of vitamin D.

TABLE 1 also shows the HLA typing, delay diagnostic, endoscopic and histological findings at diagnosis and after GFD. After one year of GFD, eight patients were reevaluated. Adherence was report by all, referring improvement of symptoms, with return of them only when gluten was ingested inadvertently. All the laboratory tests were normal. Duodenal biopsies were done in five cases, showing histological improvement in four cases.

DISCUSSION

This study presented a series of cases of SNCD, demonstrating that, although not frequent, can occur in the clinical practice. Clinical manifestations of CD have a wide range of symptoms and some patients can show oligo symptomatic form, and the serological tests are useful for identifying CD. The SNCD is characterized by the lack of demonstrable serological markers along with clinical signs of malabsorption and atrophy of the mucosa in patients ingesting gluten[6]. Even with a high sensitivity of tests, a minority of patients with CD are seronegative, with prevalence of 1.03% among all CD patients to 28% in latent CD[7].

All diagnostic serologic testing should be done with the patients on a gluten-containing diet[2]. It is recommended that cases of patients ingesting gluten with negative serology, but with positive histology for CD and compatible HLA, may be treated as having CD[8].

In CD, the production of autoantibodies occurs in the intestinal mucosa, as evidenced by the presence of immune complexes by immunofluorescence test[7]. These autoantibodies usually cross the mucosa and reach the blood vessels. However, in SNCD these antibodies may stay in the lamina propria rather than passing into the bloodstream[7]. Immaturity of plasma cells is another hypothesis in the mechanism of SNCD[9].

In this study, we found in all cases HLA compatible with CD, supporting the diagnosis of CD[10]. CD occurred in subjects who presented HLA-DQ2 and/or DQ8 gene loci, at any age, following ingestion of gluten-containing food[10]. DQ2 is detected in 95% and DQ8 in 5% of the patients with CD, and although these haplotypes are very common in the general population, with a mean prevalence of 20%, although only a minority develop CD, presenting a high negative predictive value[10]. Thus, in SNCD, HLA testing is useful to support diagnosis when biopsy or serology are equivocal.

The symptoms referred by the patients were similar to the reported in Brazilian patients with seropositive CD[11]. Nutritional profile of adults with CD is important, however, in our patients 60% presented normal weight, 10% were above and 30% present overweight. Awareness of obesity needs to be considered to avoid fail or delayed in the diagnosis of CD[12].

Anemia due to iron and vitamin B12 deficiency was verified in 40% of our patients and low levels of vitamin D in all, with bone disease in four, inclusive two young patients. Deficiency of micronutrients could require dietary supplementation in the beginning of treatment until a strict GFD proved improvement[13].

In Brazilians patients with seropositive CD, Silva et al. demonstrated that 69% at diagnosis had low bone mineral density, being 36 (35.6%) younger than 30 years[14], values similar to observed in the present investigation, which low bone mineral density was detected in four cases.

Although all of our patients had symptoms of CD, the observed diagnostic delay was long in almost all cases. The fact that serological tests are negative can influence this fact, given that such tests are used as screening. In patients with SNCD, the difficulty in diagnosing CD is certainly greater and further investigation is necessary.

There was not strict correlation between endoscopy and histologic findings since duodenal mucosa can show patchy lesions[15]. Our findings are in accordance with this proposition.

Clinical and laboratory parameters improve in patients with SNCD after a strict adherence to a GFD, similarly to patients with seropositive CD, as was find in our study. Especially important is the improvement of intestinal mucosa observed in the most of patients in the duodenal specimens obtained after GFD as demonstrated in the present study. Histological changes after a GFD in patients with and without villous atrophy strongly support diagnosis of SNCD[2].

Immune mediated disease (IMD) are reported as comorbidities in patients with CD before, at diagnosis or in the follow-up[15]. In the present study, we confirm this fact. Before the diagnosis of CD, one man reported hypothyroidism after Hashimoto thyroiditis, scleroderma in plaques and alopecia areata. After a GFD, three patients presented immune mediated disorders in a one-year reevaluation (two endometriosis, one hyperthyroidism and one lymphocytic colitis). If diagnosis of CD and subsequent dietary treatment can prevent autoimmune diseases remains controversial[16].

This study had some limitations. Three patients did not perform the second biopsy. It is need to considerer that in clinical practice is difficult that patients with an excellent response to a gluten-free diet agree to be submitted to a second upper endoscopy.

Concluding, patients presenting clinical profile of CD with negative serology and normal levels of IgA, especially those with family members with CD, should be submitted to duodenal biopsies to look for histological findings.

Authors' contribution

Kotze LMS, Utiyama SRR and Nisihara R conceived and carried out the study. Kotze LMS, Kotze LR and Nisihara R collected data. Kotze LR and Nisihara R organized and analyzed data. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Kotze LMS, Utiyama SRR, Kotze LR, Nisihara R. Doença celíaca soronegativa em pacientes brasileiros: uma série de casos. Arq Gastroenterol. 2021;58(2):214-6.

RESUMO – **Contexto** – A doença celíaca (DC) é uma doença autoimune caracterizada por reação imune principalmente ao glúten do trigo. O diagnóstico é baseado em achados clínicos, sorológicos e histológicos em pacientes que ingerem glúten. Casos em que o perfil clínico indica DC e os autoanticorpos são negativos trazem um dilema para o profissional, como o risco de não realizar ou atrasar o diagnóstico da DC. **Objetivo** – Mostrar a importância do diagnóstico correto de casos com doença celíaca soronegativa (DCSN). **Métodos** – Dez casos de pacientes brasileiros com DCSN foram estudados retrospectivamente (2013 a 2019). Foram compilados dados de queixas clínicas, autoanticorpos, níveis séricos de IgA, achados histológicos e HLA-DQ2 / DQ-8. Densitometria, atraso no diagnóstico, doenças autoimunes prévias e histórico familiar de DC também foram verificados. **Resultados** – Todos os pacientes com DCSN apresentaram sintomas clínicos de DC, com diagnóstico confirmado por achados histológicos da mucosa duodenal e positividade para HLA-DQ2 e/ou HLA-DQ8. Todos os pacientes apresentavam níveis normais de IgA e autoanticorpos negativos (IgA-anti-transglutaminase e anti-endomisial). A densitometria detectou osteopenia em duas mulheres e osteoporose em dois homens, todos com baixos níveis de vitamina D. O atraso no diagnóstico variou de 1 a 19 anos. A ocorrência familiar de DC foi relatada em 40% dos casos. Após 1 ano de dieta isenta em glúten, oito pacientes referem melhora dos sintomas, enquanto as biópsias duodenais, realizadas em cinco casos, mostraram melhora histológica. **Conclusão** – Pacientes que apresentam quadro clínico de doença celíaca com sorologia negativa e níveis normais de IgA, principalmente aqueles que possuem familiares com doença celíaca, devem ser submetidos à biópsia duodenal para pesquisa de achados histológicos.

Palavras-chave – Doença celíaca; auto anticorpos; diagnóstico.

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