Coeliac disease – a review of concepts and new developments

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Abstract

Celiac disease is a chronic enteropathy which continues to present a challenge for the clinic and for investigation, due to its atypical manifestations and etiopathogenic complexity. Studies in recent years in this area have led to the detection of silent forms, and the discovery that the real prevalence of the disease is much higher than initially supposed.

The intention of this work is to review the clinical, epidemiological and physiopathological concepts of celiac disease, updating them in light of the latest advances by important research centers, particularly in the field of genetics. A schematization is suggested in the approach to the disease, based on the latest developments.

A survey was carried out of articles in the Medline/Pubmed/Medscape databases, and specific addresses on the World Wide Web for medical journals published in recent years, searching for review articles and research works on celiac disease. A literature review was also carried out of national articles in the Índex Revistas Nacionais Portuguesas, and an analysis of text books and the register of conclusions of scientific congresses on celiac disease.

Key words: celiac disease, gluten, immunity, genetics, investigation.

Introduction

Celiac disease is an immune-mediated enteropathy that develops in susceptible individuals as a result of the ingestion of gluten, a protein contained in wheat flour and other cereal flours. In these individuals, ingestion of this protein leads to infiltration of the intestinal mucosa by intraepithelial lymphocytes CD8+ and CD4+ of the lamina propria, leading, in severe cases, to cryptal hyperplasia and villous atrophy.[1]

The inflammatory process triggered can lead to malabsorption of various important nutrients. Clinical recovery and restoration of the intestinal mucosa, following the commencement a gluten-free diet, are the main objectives following diagnosis.[2]

Celiac disease continues to present a challenge for physicians, and is clearly more prevalent than initially suspected.[3] A great deal of research effort has been dedicated to identifying the genetic factors that predispose to the disease, and the environmental agents that can trigger it.

Epidemiology

Approximately a decade ago, celiac disease was considered a relatively uncommon entity, and epidemiological data are found which refer to a prevalence of 1/1000. However, recent studies have demonstrated a much higher prevalence, and it is currently estimated that celiac disease may affect 1 in every 200 individuals. The image of the tip of an iceberg was proposed at the Seventh International Congress on celiac disease, to explain the epidemiology of the disease *(Fig.1)*. According to this scheme, the disease is clinically recognized in just a minority of patients, which explains the inaccuracies of previous studies on its prevalence. The majority of patients have an asymptomatic form, known as *silent celiac disease,* which makes diagnosis more difficult.

In the last few years, various international studies have been published on the incidence and clinical spectrum of the disease,[4-12] including that of Sanders *et al,* carried out in the United Kingdom, which confirms the existence of a large population of patients with

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silent celiac disease, detected in a primary health care population sample, which also reveals the association between celiac disease and irritable bowel syndrome. That study included a sample of 1200 patients, with serological detection and confirmation by biopsy of 12 new cases (1%); 123 patients (10.3%) met the ROME II criteria for irritable bowel syndrome and of these, 3.3% were diagnosed as having celiac disease.

In Portugal, prevalence of the disease is similar to that of the rest of Europe (1/130-300).[10] Casuistics have been published by some institutions, which a clear emphasis on pediatric services but also focusing on celiac disease in adults,[13,14] in this case with particular reference to the silent forms of the disease.

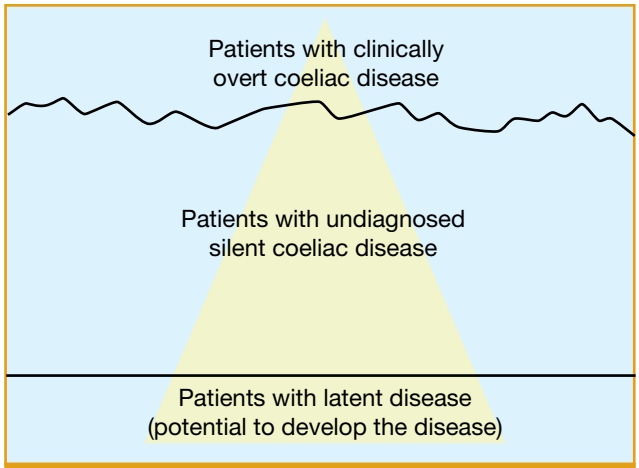
Celiac disease can occur at any age, but in adults, incidence peaks in the fifth decade. Prevalence is higher among females, and among women of fertile age, the female to male ratio is 3:1.[15] Although celiac disease is classically related to infancy, it is evident today that celiac disease in infancy is becoming progressively less common.[16,17] This fact may be partially due to the exclusion of gluten from children diet, which became common practice in various countries from the 1970s. It is also speculated that children with susceptibility for celiac disease may only develop the disease later in life, after exposure to precipitating factors.[2]

Etiopathogeny

Recent studies have defined more clearly not only the structural base of gluten intolerance in celiac disease, but also its genetic, immunological and biochemical bases.

The etiopathogenic base of the disease is the inflammatory process caused by the inappropriate immune response of the intestinal T-cells, which react to the gluten peptides. The toxic fraction of wheat gluten is gliadin. Prolamines, which are structurally similar to gliadin, are found in the gluten of other cereals. Gliadin is an alcohol-soluble component, with four subfractions in electrophoresis: α, β, γ and ω-gliadin, with varying degrees of toxicity for celiacs.[18]

The T-cells respond to these peptides, which are linked to the predisposed haplotypes of the disease - HLA-DQ2 or HLA-DQ8 - releasing IFN- γ, which is presumably what causes tissue damage. Various T-cell-stimulating peptides are known, and it is now clear that there are various sequences that can stimulate the T-cells.[19,20]



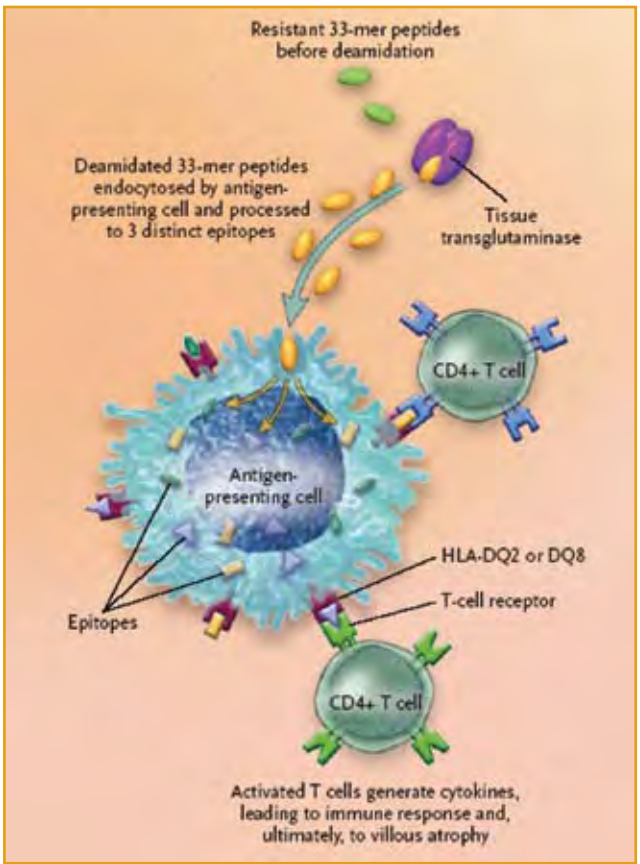
Iceberg model for Celiac Disease prevalence (Conleth Feighery, Revisão quinzenal: Coeliac Disease, BMJ 1999;319:237)

FIG. 1

The peptides recognized by the T-cells become more immunogenic after deamination, a process in which specific glutamine residues are selectively converted to glutamic acid, an action mediated by the tissue transglutaminase enzyme (tTG), which is anti-endomysial antibody antigen. These facts make clear the existence of a central action of this enzyme in the pathogeny of the disease, and for this reason, its action has been exhaustively studied.[21]

The ingested proteins, responsible for celiac disease, are digested by the proteolytic enzymes in the digestive tube, but it has been demonstrated that a sequence of 33 amino acids (33-mer) remains intact during the digestive process. This sequence has high affinity for the tissue transglutaminase whereby, after being deaminated by this enzyme, the immune response is triggered and enters the antigen-presenting cell, whereupon the sequence is processed and linked to the HLA-DQ2 or HLA-DQ8, with subsequent recognition by the T-cell receptors of the CD4+ lymphocytes (*Fig.2*).[22] It is speculated that the digestion capacity of this sequence could represent a valid therapeutic approach to celiac disease. The recent discovery of a bacterial endopeptidase capable of achieving this, along with a possible genetic intervention, is the main hope for an alternative to the gluten-free diet.

A knowledge of intraepithelial lymphocyte biology is useful for understanding the physiopathology and clinical presentation of the disease. Intraepithelial lymphocytes are T-cells with cytolytical and immu-



Gliadin 33 sequential aminoacids triggering an immune response.
In Celiac Disease – The Villain Unmasked? Ross McManus, Ph.D., and Dermot Kelleher, M. D., NEJM 348; (25)2573-2574.

FIG. 2

noregulatory properties. They are distinct from the systemic lymphocytes and in the small intestine, more than 70% are CD8+. Two subtypes have been defined: type "a", which includes the T-cells receptor α/β+ and recognizes the Major Histocompatibility Complex (MHC) type I and II, and type "b", which includes the T-cells receptor α/β+ CD8 αα+ and the T-cell receptor γ/δ +, which respond to antigens not normally related to MHC. These lymphocytes differ substantially from one another, but they share common properties, such as their similar genetic expression.[23] The upper normal limit of the intraepithelial lymphocytes is 40/100 epithelial cells. Various studies demonstrate not only an increase of this value in celiac patients, but also alterations in the topology of its distribution in the intestinal villosities.[24] It is observed that in celiac disease, the intraepithelial lymphocytes differ from normal lymphocytes. They are more numerous, both in absolute terms and in the subtype T γ/δ. Their "natural killer" activity is reduced, and they produce higher amounts of IFN- γ and IL 10, which could increase the immune reaction, leading to higher recruitment of lymphocytes and increasing the permeability of the epithelium to new antigens.[25]

The genetic susceptibility of the disease is suggested by the high agreement between monozygotic twins (60 to 70%)[2] and by the prevalence of celiac disease among the next-of-kin of celiac patients (10%), which is as high as 30% in cases of identical HLA. It is believed that celiac disease is a pathology of polygenic etiology, the most important genetic factor of which is HLA.[22] The association between HLA and celiac disease is explained by the observation that the HLA-DQ molecules present gluten peptides to the T-cells. This association between celiac disease and HLA has been exhaustively studied, and it is verified that around 95% of celiac patients are carriers of the alleles DQA1*0501 and DQB1*0201, responsible for codifying the HLA DQ2 type. Of the remaining patients, the majority do not present this pattern, and are carriers of the alleles DQA1*0301 and DQB1*0302, which codify the HLA DQ8 type.[26] The practical implications of these conclusions are important, because although research has found that the haplotypes HLA DQ2 and HLA DQ8 are not determinants for diagnosis of the disease, since they are present in the general population at levels of 25% and 32%, respectively,[27] they are nevertheless extremely useful for ruling out the disease, since celiac patients who are negative for DQ2 and DQ8 are extremely rare.

It is believed that in patients with celiac disease with the HLA DQ2 pattern, the inflammatory response of the T-cells to the gluten peptides linked to the HLA-DQ2 is responsible for the disease. There are two types of HLA-DQ2 molecule; HLA-DQ2.5 and HLA-DQ2.2. While HLA-DQ2.5 predisposes to the appearance of the disease, HLA-DQ2.2 does not. The presence of the homozygote or heterozygote for type HLA-DQ2, as well as the presence of the homozygote for type HLA-DQ2.5 or the heterozygote HLA-DQ2.5/2.2, are responsible for the variable severity of the disease.[28]

All the studies to date demonstrate an evident relation between the disease and the short arm of chromosome 6, the region responsible for the HLA.[3]

The association between other sites of the genome

and celiac disease has been the object of sporadic reports.[29-33] The association is emphasized between the atypical form of the disease, and a polymorphism that exists in exon 5 of the MICA A5.1 gene. The MICA genes are genes which are contained in the HLA 1 region of chromosome 6; MICA protein 5.1 is found in the apical region of the enterocytes, and is recognized by the T-lymphocytes ($\gamma\delta$+). The polymorphism described provokes the appearance of a soluble protein which is not linked to the membrane, whereby this could represent an alteration in the normal regulatory function of the T-lymphocytes, in the homeostasis of the intestinal epithelium.[3]

Another possible association between susceptible genomic zones and celiac disease relates to the gene responsible for codifying the cytotoxic T-Lymphocytes CTLA-4 ("cytotoxic T-Lymphocyte associated protein-4"), located at 2q33. The appearance of polymorphisms in exon 1 is responsible for the reduction in inhibitory function of the CTLA-4 in the autoimmune process, with an evident association between this region and the disease.[34,35]

CLINICAL PRACTICE

Signs and symptoms

The signs and symptoms of celiac disease are divided between intestinal manifestations and symptoms and signs caused by malabsorption. However, many patients, particularly those who manifest the disease in adulthood, have minimal or atypical symptoms.

The symptoms that classically suggest a diagnosis of celiac disease are abdominal distension, chronic diarrhea and weight loss.[27, 36]

In children under 2 years, a more aggressive form of the disease is possible, and symptoms may include chronic diarrhea, retarded growth, abdominal distension and vomiting.[2] Nowadays, this clinical presentation is less common, and pediatric patients tend to manifest the disease at a later age, around 4 years, mostly with more subtle manifestations, such as low stature and loss of appetite.[2]

The symptoms of intestinal disorder may be absent in adults with celiac disease. In clinical cases with typical manifestations of the disease, oral ulcers, dyspepsia and alterations in tooth enamel are typical, and these may be the only form of manifestation of the disease.

Subclinical nutritional deficiencies are frequent, and mineral bone density may be altered, even in childhood.[37] A wide spectrum of malabsorption may be established, including anemia, due to iron and/or folic acid deficiency, and less frequently, cobalamin deficiency. The levels of serum calcium and liposoluble vitamin D, and less frequently, vitamin K, may be low.[36]

Neurological alterations are common in celiac disease in adulthood, and include the presence of peripheral neuropathy, memory loss and ataxia.[36] Alterations may occur in the reproductive system; in women, specific disorders may occur, such as delayed menarche, amenorrhea, early menopause and even infertility; in men, sexual impotence, decreased sexual activity and morphological and functional alterations of the spermatozoids[27] are also common. In addition to the extra-intestinal manifestations described above, a series of pathologies has indicated out in association with celiac disease, as described below.

Associated diseases

An increase in the prevalence of celiac disease is seen in association with certain types of pathology, such as type 1 diabetes and autoimmune thyroid disease. The possibility of association with other autoimmune entities, such as primary biliary cirrhosis and Sjögren's syndrome, appears to exist, but in a less apparent form. The common genetic basis, in particular type HLA, and the existence of common mechanisms of mediated immune disease, may be the basis of these associations.[2]

The sharing of a similar HLA haplotype may explain, in part, the strong association between IgA deficiency and celiac disease. [27, 2]

Herpetiform dermatitis is an erythematous-vesicular cutaneous disease which is characterized by the presence of granular deposits of IgA in the basal membrane of the skin. Having demonstrated that these legions may be manifestations of skin intolerance to gluten, researchers nowadays consider this entity to be a manifestation of celiac disease, and not an associated disease.[27,36]

It is suspected that untreated celiac disease may be related to long-term neoplasic complications, such as squamous cell carcinoma of the esophagus, small intestine adenocarcinoma, and even enteropathic T-cell lymphoma. Following a gluten-free diet for a period of five or more years appears to eliminate the risk of the appearance of these neoplasias.[18]

TABLE I

Celiac disease sensitivity and specificity in serological tests

	Sensitivity (%)	Specificity (%)
Antigliadin antibody IgA	87	85
Antiendomysium antibody IgA	93	100
Anti-tissue transglutaminase antibody IgA	95	95

Complementary diagnostic methods

In celiac disease, analytical alterations are frequent, the most usual ones being low hemoglobin, albumin, calcium and potassium levels, due to malabsorption associated with the disease.

Damaged cells and Howell-Jolly bodies are present in the peripheral blood smear in around 50% of patients with celiac disease in adulthood, and are related to splenic hypofunction. Celiac disease is the most frequent condition associated with hyposplenism.[27]

The method of choice for establishing a diagnosis of celiac disease continues to be intestinal biopsy, preferably performed twice; before and after the institution of a gluten-free diet.

With the notion of high prevalence of hidden forms of celiac disease, serological tests are currently frequently carried out. Various serological antibody tests have been developed to select candidate patients for intestinal biopsy. The anti-reticulin and antigliadin antibodies were the first tests to be used for screening, and the latter are still widely used. However, in the context of screening for celiac disease in asymptomatic patients and various at-risk groups, the benefits of the most recent IgA anti-endomysial antibody, and the last, tissue *anti-transglutaminase,* are obvious *(Table I)*.[36]

The specificity of these tests is close to 100% and they have sufficiently high sensitivity for the purposes of screening. The antiendomysium antibody is an immunofluorescence test, while the tissue *anti-transglutaminase* is based on ELISA, and is therefore easier to interpret and more appropriate for larger-scale screening programs.

A positive test for these antibodies should always be confirmed by intestinal biopsy.

Given that around 2 to 3% of patients with celiac disease show selective IgA deficiency, these antibodies remain negative, therefore the IgG antigliadin antibodies and total serum IgA may be used for screening in these cases.[36]

In the endoscopy, the appearance of the intestinal mucosa varies, depending on the severity of the disease. Latent celiac disease typically does not show thickening of the mucosa, and the existence of normal intestinal folds is seen. In the clinically active disease, there may be nodulation, known as scalloping. In the severe form of the disease, the folds are smaller in size and number, or may be completely absent. A mosaic pattern of the blood vessels may also be evident in severe celiac disease.[18]

Various histological alterations are found in the intestinal biopsy of patients with celiac disease. An increase in intraepithelial lymphocytes (> 40/100), as described above, is the first and most significant effect of gluten on the intestinal mucosa, and is therefore the main histological aspect of celiac disease. There is an increase in cellularity of the lamina propria, with an increase in the number of plasma cells, lymphocytes and eosinophils, particularly in the upper region of the mucosa. In severe celiac disease there is visible damage of the enterocytes, with cytoplasmatic vacuolation, decreased size, and easy dislocation of the basal membrane. Atrophy of the villosities and hyperplasia of the crypts represent severe damage of the intestinal mucosa. Marsh's classification of celiac disease interprets the histological alterations detected and relates them to the spectrum of the disease *(Table II)*.[18]

A spectrum of new tests is currently being investigated, in order to provide more advanced means of diagnosing and screening for the disease, namely, new serological targets with four recently identified auto-antigens (actin, chain β of the ATP synthase, and two enolase α variants).[38] The serum motilin and IL-18 levels appear to increase in the presence of celiac disease. The level of nitric oxide in the urine, which increases in response to intestinal inflammation, is promising as a quick and easy screening test for the disease. Other auxiliary diagnostic texts, such as the sorbitol breath test, and imaging methods, such as doppler of the splanchnic vessels, are awaiting consolidation of the results before their usefulness can be determined for the diagnosis of this entity.[26]

Treatment

The treatment of celiac disease is based on the following points: starting a gluten-free diet, monitoring

TABLE II

Classsificação de Marsh da doença celíaca

Marsh type	Intraepithelial lymphocytes	Crypt	Villosities
0	<40	Normal	Normal
1	>40	Normal	Normal
2	>40	Enlarged	Normal
3a	>40	Enlarged	Moderated atrophy
3b	>40	Enlarged	Marked atrophy
3c	>40	Enlarged	Absent

Type 0: normal mucosa, unlikely celiac disease

Type 1 (infiltrating lesion): seen in patients on free gluten diet, patients with Dermatitis Herpetiformis and Celiac Disease patients relatives; these patients need close monitoring, as they may evolve to type 3.

Type 2 (hyperplasia type): very rare, occasionally seen in Dermatitis Herpetiformis patients.

Type 3 (destructive lesion): range of impairments seen in symptomatic patients.

clinical progress, ensuring the regular support of a dietician, providing nutrient supplements where necessary, such as iron, folic acid, and calcium, monitoring compliance to the diet with seriate antibody tests and repeating intestinal biopsy if the clinical evolution is not sufficient.[2]

Definitive exclusion of gluten from the diet is the standard treatment. Besides foods containing wheat, foods which contain rye or barley are also excluded. Despite the exclusion of wheat, there is increasing evidence that this may not be toxic for celiac patients.

Specialized follow-up is advised when giving guidance on diet, despite the usefulness for patients of information published by associations for celiac sufferers.

Many patients show a dramatic initial clinical response, and improvement of the symptoms within a few weeks. Histological recovery is slower, and complete recovery of the mucosa may take months or even years.[2]

In rare cases, immunosuppression therapy may be necessary for forms of the disease that do not respond to a gluten-free diet.[27] In this case, corticoids are used, or in more severe forms, cyclophosphamide or azatioprin.

Although no alternative cure to the gluten-free diet has yet been found, new and recent scientific developments bring some hope, particularly the discovery, mentioned earlier, of an enzyme – propyl endopeptidase– capable of breaking down the immunostimulant gliadin peptides (33-mer).

When that action is seen, the basic mechanism of the etiopathogeny of the disease is annulled, which could represent a unique therapeutic option. Advances in understanding of the etiopathogeny of the disease, particularly in relation to its immune mechanism and genetic base, have led to high expectations of immunotherapy and genetic intervention as future therapeutic weapons. ■

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