

CELIAC DISEASE - GENERAL AND LABORATORY ASPECTS

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5.1 Introduction

Celiac disease is a chronic immune-mediated disorder in genetically susceptible individuals induced by gluten proteins present in wheat, barley, and rye. Celiac disease is associated with HLA DQ2 and DQ8. It is a common lifelong disorder characterised by a variety of clinical presentations, among which are gastrointestinal symptoms and anemia. The prevalence of the disease (symptomatic and asymptomatic) is estimated to be close to 1%. Anti-transglutaminase IgA (or anti-endomysial IgA) in combination with total serum IgA is the preferred screening test for identifying individuals who need to undergo endoscopic duodenal biopsy examination., which is needed for definitive diagnosis. The pathologic lesion is characterised by a flattened small intestinal mucosa with a lymphocytic infiltrate, crypt hyperplasia, and villous atrophy. Untreated celiac disease is associated with increased morbidity and mortality (due to gastrointestinal tract malignancies). The pathologic changes and symptoms resolve when gluten is excluded from the diet.

5.2 Gluten

Celiac disease is triggered by ingestion of wheat gluten or related proteins present in rye and barley. Gluten is the water insoluble seed storage protein in wheat. Gliadin is the alcohol soluble component of gluten, and is the most toxic component of gluten. Gluten and related proteins in rye and barley are responsible for the favourable cooking and baking properties of these grains.

5.3 Clinical presentation

The clinical manifestation of celiac disease is variable and may involve multiple organ systems. The disease may present at any age. Delays in diagnosis are common.

The classic presentation associated with celiac disease is characterised by steatorrhea, abdominal distention, edema, and extreme lethargy. Nowadays, diarrhoea occurs in less than 50% of patients at presentation. Also weight loss is now a less common feature than in the past and is associated with extensive disease. In some patients, overweight is seen at the time of diagnosis. Abdominal pain, bloating, distention, anorexia, constipation, and altered bowel habit may occur in the absence of malabsorption.

In the atypical form of celiac disease, which is now common, gastrointestinal symptoms may be less pronounced or absent and extraintestinal features are more prominent. It is important that the atypical features of celiac disease are recognised.

A distinctive example of an extraintestinal manifestation is dermatitis herpetiformis. This disease is characterised by a pruritic rash on the elbows, knees, buttocks, and scalp and the presence of granular IgA deposits in the dermal papillae.

Iron deficiency anemia is a common finding in celiac disease and may be the only presenting sign. Iron is absorbed by the proximal small intestine, the site of the greatest damage in celiac disease. Besides, there might be a deficiency of vitamin D, vitamin B12, and folate. Other presentations are unexplained short stature and developmental delay in children, infertility and recurrent fetal loss, recurrent mouth ulcers, dental enamel defects, osteoporosis, fatigue, protein calorie malnutrition, and elevated transaminases.

Celiac disease may also be associated with autoimmune disorders (type I diabetes, autoimmune thyroid disorders, Addison disease Sjögren's syndrome, primary biliary cirrhosis, autoimmune hepatitis, autoimmune cholangitis), and cardiac disorders (autoimmune myocarditis, idiopathic dilated cardiomyopathy). Celiac disease has also been reported in patients with unexplained neurological complaints (cerebellar ataxia, neuropathy, epilepsy, migraine).

Genetically associated diseases include IgA deficiency, Down syndrome, Turner syndrome, and Williams syndrome.

Some individuals may have no symptoms at all and can be labelled as having silent or asymptomatic celiac disease. These patients have villous atrophy that may be discovered during intestinal biopsy for other reasons, or as a result of serologic screening of high-risk individuals. Individuals who are antibody (IgA anti-endomysial) positive but with normal or minimally abnormal small bowel biopsy examination, have been described as having latent or potential celiac disease.

5.4 Complications of celiac disease

All cause mortality among patients with clinically diagnosed celiac disease is about 2 times that of the control population. The increased mortality has been attributed to gastrointestinal tract malignancies, especially intestinal non-Hodgkin lymphoma. Enteropathy-associated T-cell lymphoma is rare and occurs in adult patients with celiac disease. Some evidence suggests that a gluten-free diet may reduce lymphoma risk. There is also an increased risk of adenocarcinoma of the small intestine, the pharynx, and the esophagus. The increased mortality has been associated with delayed diagnosis.

5.5 Prevalence of celiac disease

There is scarcity of data on the incidence of the full spectrum of celiac disease, including classical, atypical, silent and latent forms. Recent studies using serology and small intestinal biopsy suggest that the prevalence of celiac disease in Europe and in the United States is 0.5%-1%. This included both symptomatic and asymptomatic individuals. There is probably a substantial number of undiagnosed cases in the general population (possibly 10 times as many as actually have been diagnosed).

Certain populations have an increased prevalence: first degree relatives (4%-12%), type I diabetes mellitus (3%-8%), and Down syndrome (5%-12%). Furthermore, celiac disease is associated with i.a. IgA deficiency and autoimmune disorders.

5.6 Genetic factors

Genetics clearly play a role in the pathogenesis of celiac disease. The presence of specific alleles at the DQ locus appears to be necessary, although not sufficient, for the phenotypic expression of the disease. HLA-DQ2 or -DQ8 is present in almost all individuals with celiac disease. DQ2 is present in approximately 90%-95% of celiac disease patients and DQ8 in the remaining 5%-10% of patients. The DQ2 heterodimer that confers celiac disease susceptibility is formed by a β chain encoded by the allele DQB1*02 (either DQB1*0201 or 0202) and a α chain encoded by the allele DQA1*05. The HLA-DQ8-associated heterodimer is formed by a β chain and α chain encoded by DQB1*0302 and DQA1*03, respectively.

The presence of HLA-DQ2 or DQ8 is not helpful as a positive predictor of disease, as about 30% of the general population has HLA-DQ2 or DQ8 and only about 1:30 people with these genes have celiac disease.

5.7 Antibody markers

Anti-gliadin and endomysial antibodies are associated with celiac disease and are helpful in the diagnosis and management of the disease. Although anti-reticulin antibodies were used formerly, they have been replaced by the anti-endomysial antibody test in many laboratories. The anti-endomysial antibody test is an indirect immunofluorescence test. As substrate, human umbilical cord or monkey esophagus is used. In 1997, tissue transglutaminase was identified as the target antigen of the anti-endomysial antibodies.

Since then, ELISA systems for detection of anti-endomysial antibodies have been developed. In the first generation ELISAs, guinea pig tissue transglutaminase was used whereas the second generation ELISAs are based on the use of human recombinant antigen.

The diagnostic performance of the serologic markers for celiac disease varies depending on the study. Recently, a systematic and rigorous review of the literature, in which only studies that used biopsy as the gold standard were included, has been published (Hill ID, *Gastroenterology* 2005; 128: S25-S32).

Table 1 summarises the sensitivities and specificities reported in this study for the various antibodies for celiac disease. The IgG anti-gliadin antibody has a sensitivity and specificity of about 80%. IgA anti-gliadin antibody has a sensitivity that is comparable to the IgG anti-gliadin antibody, but the specificity is higher. Anti-endomysial IgA and anti-human recombinant tissue transglutaminase IgA are the most sensitive (> 90%) and specific (> 95%) serologic tests. Testing for IgG-anti-gliadin, IgG anti-transglutaminase, IgG-endomysial antibodies is useful for diagnosing celiac disease in IgA-deficient individuals.

Table 1. Sensitivity and specificity of anti-gliadin IgG, anti-gliadin IgA, anti-endomysial IgA, and anti-tissue transglutaminase IgA for the diagnosis of celiac disease. The data are obtained from Hill ID (Gastroenterology 2005; 128: S25-S32) who reported a systematic review of articles written from 1966 to 2003. Inclusion in the systemic review required that diagnosis of celiac disease was confirmed by biopsy and that control individuals had normal histological findings on small bowel intestinal biopsy examination.

Marker	Number of studies	Sensitivity	Specificity	
Anti-gliadin IgG	17	57%-100%	47%-100%	Sens > 90% in 7/17 studies
				Spec > 90% in 3/17 studies
Anti-gliadin IgA	26	52%-100%	71%-100%	Sens > 90% in 7/27 studies
				Spec > 90% in 19/26 studies
Anti-endomysial IgA	32	86%-100%	90%-100%	Sens > 95% in 17/32 studies
				Spec > 95% in 31/32 studies
Anti-tissue transglutaminase IgA*	22	77%-100%	91%-100%	Sens > 95% in 16/22 studies
				Spec > 95% in 18/22 studies

**Assays based on guinea pig antigen and human recombinant antigen. It should be mentioned that assays based on human recombinant antigen are more sensitive and more specific than assays based on guinea pig antigen.*

5.8 Diagnosis of celiac disease

It is important that clinicians are aware of and recognise the clinical spectrum of celiac disease. All diagnostic tests need to be performed while the patient is on a gluten-containing diet. The first step should be a serologic test. Anti-human recombinant tissue transglutaminase IgA or anti-endomysial IgA are the most accurate serologic tests. It should be mentioned that anti-tissue transglutaminase (and anti-endomysial) antibodies may miss a rare patient with celiac disease. It is helpful to also measure total IgA. If IgA deficiency is found, measurement of IgG anti-gliadin or IgG anti-tissue transglutaminase (or endomysial) antibodies is recommended. The performance of serology in children younger than 5 years is less well known and requires further study.

Biopsies (at least four) of the proximal small bowel (second part of duodenum or beyond) are indicated in individuals with positive serology. In an individual with suggestive symptoms and a negative serology test, an alternative serologic test should be performed and/or a small intestinal biopsy conducted. The characteristic histological findings are

blunted or flat villi (villous atrophy), hyperplastic crypts, loss of surface enterocyte cell height, and a lymphocytic infiltration of the lamina propria. Some degree of villous atrophy is necessary to confirm a diagnosis of celiac disease. Although not used universally, the Marsh classification can be applied to standardise the pathology reports. A Marsh I lesion indicates a lymphocytic infiltration with normal mucosal architecture. Marsh II lesion exists when there is, in addition to a lymphocytosis, crypt hyperplasia. Marsh III lesion is characterised by villous atrophy. Marsh IV lesion is a rare finding and is associated with refractory disease and development of enteropathy-associated T-cell lymphoma.

A presumptive diagnosis of celiac disease can be made based on concordant positive serology and positive biopsy results. Definitive diagnosis is confirmed when symptoms improve with a gluten free diet. The serum antibodies generally disappear by 6 to 12 months, although they do not reliably reflect mucosal response. Many clinicians consider that demonstration of normalized histology following a gluten-free diet is no longer required for a definitive diagnosis of celiac disease. However, demonstration of histologic improvement can assure the diagnosis in patients who did not present with classical clinical features. Gluten challenge is not performed routinely now, unless there is a diagnostic difficulty, for example in patients who were already on a gluten free diet but in whom no initial diagnostic biopsy was performed.

Demonstration of the characteristic abnormalities on biopsy is key to the diagnosis of celiac disease. If histologic examination yields equivocal results, HLA typing can be useful. A negative result for HLA-DQ2 or HLA-DQ8 has an excellent negative predictive value for the disease.

5.9 Who should be tested for celiac disease?

Patients who present with the classical gastrointestinal symptoms of celiac disease and/or with atypical symptoms of celiac disease (see above) should be tested. Besides, symptomatic individuals in populations at higher risk for celiac disease (e.g. autoimmune endocrinopathies, first- and second degree relatives of individuals with celiac disease, Down syndrome) should also be tested for celiac disease. Because current data do not indicate a outcome benefit of early detection and treatment of asymptomatic individuals in these groups, routine screening cannot be recommended at this time. Similarly, there are insufficient data to recommend screening of the general population for celiac disease at this time. The long-term benefits of early detection of celiac disease and treatment with a gluten-free diet in asymptomatic individuals are not proven.

5.10 Pathogenic mechanism

Dietary gluten proteins in wheat and similar proteins in rye and barley are the triggers of celiac disease in individuals with the disease susceptibility HLA-DQ2 and HLA-DQ8 alleles. Based on the current knowledge, the following model can be put forward. Gluten peptides that are rich in proline and glutamine and not fully digested by gastric and pancreatic enzymes reach the small intestinal mucosa, most probably because of increased intestinal permeability, as can occur after gastrointestinal infection. The glutamine-rich gluten peptides are deamidated by tissue transglutaminase, which is released during tissue repair associated with infection. This tissue transglutaminase-

driven modification is an important step in the immune response in celiac disease as the resulting deaminated and thus negatively charged peptides have a high affinity for HLA-DQ2 and HLA-DQ8 molecules (on dendritic cells, macrophages, and B cells) that are involved in presenting these peptides to CD4(+) T cells. Recognition of HLA-bound gluten peptides by CD4(+) T cells leads to their activation and release of cytokines. The cytokines (i) induce an inflammatory response (with release of matrix metalloproteinases that cause epithelial cell damage) and (ii) activate the production of antibodies by B lymphocytes. In celiac disease, CD8(+) and CD4(-)CD8(-) T cells infiltrate into the epithelium and probably play a role in lymphocyte-mediated destruction of epithelial cells and mucosal damage. The resulting tissue injury leads to further release of tissue transglutaminase.

In addition to having deaminating activity, tissue transglutaminase also has cross-linking activity. This cross-linking activity of tissue transglutaminase is involved in various functions, such as wound healing and stabilisation of the extracellular matrix (e.g. by cross-linking of collagen molecules). Tissue transglutaminase is therefore expressed during tissue injury. Its expression is elevated in intestinal biopsy samples from patients with celiac disease. Tissue transglutaminase can form covalent complexes with gliadin, due to its cross-linking activity. The anti-tissue transglutaminase immune response might be generated by epitope spreading through intermolecular help, where gliadin acts as a carrier protein for tissue transglutaminase. The role of the antibodies to the mucosal lesions is not clear.

5.11 Treatment of celiac disease

Patients should not start a gluten-free diet until a definite diagnosis has been reached. Treatment of celiac disease is strict lifelong adherence to a gluten-free diet. Food products containing wheat, rye, or barley must be avoided. Grains that can be used for substitution include rice, corn, quinoa, and buckwheat. Adherence to a gluten-free diet is difficult as wheat flour is ubiquitous in foods. A dietician should be consulted.

Literature

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