BIOCHEMICAL DIAGNOSIS OF CELIAC DISEASE

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ABSTRACT
Celiac disease is a chronic inflammatory condition associated with small intestinal injury that results in the malabsorption of different nutrients. The damaging factor is gluten present in wheat, barley and rye. The diagnosis relies on the clinical picture of the patient, serological markers for celiac disease, characteristic findings of small intestinal biopsy and, eventually, clinical improvement on a gluten-free diet. Our strategies for the diagnosis of celiac disease have changed dramatically within the last 10 years. The advent of serological markers with high sensitivity and specificity is changing our understanding of the disease and its prevalence. Treatment includes a life-long gluten-free diet to prevent the recurrence of symptoms and other potential consequences. Most celiac disease remains under-diagnosed; the utilization of more accurate serological tests and a greater awareness of its many presentations will aid its identification.

KEYWORDS: gluten, barley, HLA-DQ2, CD4 T cells.

INTRODUCTION
Celiac disease is a condition in which there is an abnormal proximal small intestinal mucosa that improves morphologically on treatment with a gluten-free diet and relapses if gluten is re-introduced.\(^1\) The relation between the disease and wheat was first reported after World War II. Dick observed that the ingestion of certain cereal grains, including wheat and rye, was harmful to children with celiac disease.\(^2\) It is the alcohol-soluble protein components of wheat (gliadins), barley (secalins) and rye (hordeins), collectively called prolamins, that are toxic. The ingestion of gluten in genetically susceptible individuals leads to the presentation of the alcohol-soluble protein gliadin by antigen-presenting cells, in association with the human leukocyte antigen HLA-DQ2 or HLA-DQ8, to T cells expressing α/β T-cell receptor.\(^3\) These T cells then become activated and, in turn, recruit other lymphocytes that produce interferon-γ, tumor necrosis factor-α and interleukins-4, -5, -10 and -13. These cytokines are damaging factors to small intestinal epithelium. The damaged epithelium triggers the release of tissue transglutaminase (tTG), a cytosolic enzyme. This enzyme also alters gliadin by de-amidation, which, in turn, augments gliadin presentation by HLA-DQ2 or HLA-DQ8 to the gliadin-reactive lymphocytes in the celiac gut. In addition, cytokines released by lymphocytes augment the expression of HLA-DQ2 on small intestinal epithelium, allowing more gliadin to be presented to sensitized lymphocytes. CD is one of the better-understood autoimmune diseases with key features of its immunopathogenesis and underlying genetics described.\(^4, 11, 2, 6\) It is thought to be initiated in genetically predisposed individuals by the ingestion of gluten and related proteins found in grains such as wheat, rye, and barley. The events leading to CD are thought to include luminal and early mucosal events, activation of the innate and adaptive immune systems, as well as intestinal tissue damage.\(^11, 5\) In the early stages of CD, ingested gluten (gliadin and glutenin are the major protein components of gluten) is digested by luminal and brush-border enzymes into amino acids and α-gliadin peptides that are resistant to further degradation. Partially digested α-gliadin peptides are able to cross the epithelial cells and enter the lamina propria where they are cross-linked and deamidated by tTG to produce DGP. Induction of CD4 T-cell-specific responses is thought to be initiated by DGP bound with high affinity to HLA-DQ2/DQ8 molecules expressed on the surfaces of antigen-presenting cells (APCs). Activated CD4 T cells, in addition to providing help to B cells in eliciting antibody-specific responses produce proinflammatory cytokines such as gamma interferon (IFN-γ), IL-15, and IL-17. Gliadin is also thought to stimulate the innate immune system directly through the upregulation of IL-15 in the intestinal epithelial cells. IL-15 is widely
recognized to activate intraepithelial lymphocytes (IEL) as well as upregulate MIC—A.

REVIEW

Symptoms in children are usually associated with the introduction of cereals into the diet, usually after the age of 6 months. The symptoms depend on the age of the child at disease onset. In young children, there is failure to thrive, diarrhea, vomiting, muscle wasting, abdominal distension, abdominal pain and, occasionally, constipation. In older children, the disease may present as anemia, rickets, short stature, dental enamel defects, and poor performance in school or behavioral disturbances. Two to eight per cent of children with unexplained short stature presenting to a growth failure clinic have celiac disease when either endomysial antibodies or duodenal biopsies are used to test for the condition. Treatment of celiac disease may improve growth in those treated before growth is complete. A curious pattern of dental enamel defects may be apparent to an astute dentist due to the effect of malabsorption on the development of the permanent dentition. The disease in adulthood may be either adult-onset disease or silent disease present since childhood but producing no symptoms. The most common presenting symptoms in adults with celiac disease are abdominal pain, chronic diarrhea and iron-deficiency anemia. Diarrhea may be absent in 50% of patients and steatorrhea is less common (40%) and indicates more severe disease. Patients with celiac disease may be mistakenly diagnosed as having irritable bowel syndrome. Celiac disease is frequently associated with iron deficiency. Six to ten per cent of patients referred for upper endoscopy because of iron-deficiency anemia will have celiac disease based on small bowel biopsies, even in the absence of other features suggesting celiac disease. Celiac disease can result in vitamin D and calcium malabsorption. Fifty per cent of patients with celiac disease have lactose intolerance and may already have a reduced intake of calcium. A significant prevalence of celiac disease has been reported in patients who present with osteopenic bone disease. There is diminished calcium absorption that corrects after the exclusion of gluten from the diet. Severe osteopenia was discovered in one-third of patients with celiac disease previously diagnosed as children, who were consuming a normal diet. Osteopenia occurs in those with mild symptoms at a rate greater than that in the normal population, but less than that in classical celiac disease. This diminished bone density is associated with an increased risk of fractures in patients with celiac disease. A gluten-free diet does, however, correct the bone loss in those with mild disease, and significantly improves it in those with severe malabsorption. Reduced fertility or recurrent spontaneous abortion have been reported in association with celiac disease. screening of infertile couples has revealed celiac disease at a rate greater than that expected. Male infertility may also be seen in untreated celiac disease. Infertility in both males and females may be reversible with the treatment of celiac disease.

Observation & Diagnosis

1. Blood tests for gluten autoantibodies (These are IgA based tests accurate only while on a gluten-containing diet)
   - EMA - anti-endomysial
   - TTG - anti-tissue transglutaminase
   - DGP - Deamidated Gliadin Peptide

2. A small bowel biopsy to assess gut damage. For those with suspected dermatitis herpetiformis, skin biopsies will be taken of the skin near the lesion.

Serology (Blood) Tests

There is no standardization in current tests. A number of tests, sometimes collectively referred to as the Celiac Blood Panel or Cascade, will aid the physician in diagnosis. The tests may include, but are not limited to:

1. EMA (IgA anti-endomysial antibodies)
2. AGA (IgA anti-gliadin antibodies) Some people do not produce IgA antibodies.
3. DGP (Deamidated gliadin peptide antibody)
4. tTGA (IgA anti-tissue transglutaminase)

Deamidated gliadin peptide (DGP) antibodies tests developed in 2007 in combination with Tissue transglutaminase (TTG) antibodies and have better accuracy than native gliadin antibodies. Multiplex immunoassay (MIA) measures multiple antibodies simultaneously providing with reduced turnaround time and cost. Combination testing identifies patients who are candidates for an intestinal biopsy. Test panels include AGA to determine if a person’s body makes sufficient IgA antibodies for the EMA and TTG results to be reliable. IgA deficiency is, in itself harmless.

Gene Tests

Gene tests alone are not used to diagnose celiac disease. Gene tests can only exclude the probability of developing celiac disease. Thirty to forty percent of people have the genetic predisposition to develop celiac disease. 1% of the population will develop celiac disease. Human leukocyte antigen (HLA) region DQ genes are highly represented in persons diagnosed with celiac
Accurate genetic tests for celiac disease require analysis of the configuration of both DQA and DQB genes. Other genes outside of the HLA area have and are being identified. Genetic testing may be useful for family members of a person diagnosed with celiac disease and young children whose immune system is not mature. May 2011, Digestive Disease Week - Bob Anderson, MD and collaborating researchers presented the first population study results that support the use of a combination of HLA-DQ genetic and serology tests to determine the prevalence of celiac disease. This combination test may ultimately eliminate the standard guidelines of a biopsy. The study also concluded that the cost per diagnosis can be reduced by up to 50%. This non-invasive process would be a cost effective and efficient diagnostic method appropriate for the primary care setting.

Biopsy
In the event that clinical signs and positive laboratory tests indicate probable malabsorption, a biopsy of the small intestine [jejunal] is scheduled to be performed by a gastroenterologist. In this test, a small flexible biopsy instrument is passed through a tube, down the throat, through the stomach and into the upper end of the small intestine where patchy, multiple snippets of tissue are gathered. The tube is removed and the tissue samples are examined under a microscope for signs of damage. The difference between tissue in a normal small intestine and that found in an undiagnosed or untreated celiac patient is remarkable. The normal finger-like projections (villi), which increase the absorptive surface area of the small intestine, are partially or totally flattened in a person with celiac disease. Enzymes located on the brush border are also drastically reduced. The enzymes produced at the tips of the villi breakdown carbohydrates. Lactase, the enzyme responsible for splitting milk sugar (lactose) so it can be absorbed, is an example of one of these brush border enzymes. This decrease in lactase explains why some untreated celiac patients may not be able to tolerate milk products and will have developed transient or permanent lactose intolerance. At the base of the villi elevated numbers of T-cell lymphocytes (white blood cells) are also present. The small bowel biopsy samples of persons with dermatitis herpetiformis often show similar damage.

Tolerance or Measure of Digestion/Absorption Tests
1. Lactose tolerance test
2. D-Xylose test

NOTE: At this time there is no standardization in either serological testing or intestinal biopsies.
1. DISCUSSION

Either the IgA-human tissue transglutaminase (TTG) or IgA endomysial antibody (EMA) test or a combination of both are recommended as screening tests. “Celiac disease screening/panel” on a lab requisition should include one or both of these tests. An additional test is required to measure the serum IgA concentration (explained in Question #3). They are equally accurate as screens for celiac disease in individuals who regularly eat foods that contain gluten (see glossary). In addition to the above tests, the serum IgA test is used to evaluate IgA deficiency. If your body does not make serum IgA, the TTG and EMA results are usually falsely negative. IgA deficiency occurs in 3 – 5% of individuals with celiac disease. IgA deficiency alone may cause intestinal symptoms and you should discuss with your physician the need for upper endoscopy and intestinal biopsy (see glossary). The TTG and EMA tests are about 90% accurate for individuals who make serum IgA. They are not as accurate in children under age three years. Because the tests are not 100% accurate, anyone with a negative test result and symptoms suggestive of celiac disease should talk to a physician about an upper endoscopy and intestinal biopsy (see glossary). Positive and negative results values vary between test kits from different manufacturers. Each kit includes instructions on

Villus a: 1 muscularis mucosae, 2 central lacteal, 3 mucous membrane, 4 capillary network, 5 circular muscle, 6 longitudinal muscle, 7 serosa, 8 submucosa, 9 lymphatic vessel, 10 muscular coat, 11 submucosa, 12 epithelial cell, 13 arteriole, 14 venule, 15 villus
positive and negative results. The test results should be explained on the report provided by the laboratory. Your physician should discuss these results with you. No, there is no genetic test to diagnose celiac disease. Although there are tests for the HLA DQ2 and HLA DQ8 genes, they are costly and not readily available in Canada. Since about 40% of North Americans have these genes but only 0.5-1% of the population will develop celiac disease, having the gene does not mean you will develop celiac disease. For the TTG and EMA blood tests to work properly, one must be eating gluten daily. For the genetic tests, it doesn’t matter (see question #9). Your doctor may use these tests to monitor your response to the gluten-free diet after confirmatory diagnosis. An intestinal biopsy must be performed because of the 10% possibility of a falsely positive blood test. Continue to consume at least the equivalent of one to four slices of bread containing gluten every day until your endoscopy for the biopsy to be accurate. The most widely described serologic tests for CD include antireticulin antibodies (ARA), antigliadin antibodies (AGA), and EMA, tTG, and DGP antibodies. The ARA test was first introduced as a diagnostic test for CD in 1977 and is routinely detected by indirect immunofluorescence assay (IFA) on rat tissue. These antibodies (IgG or IgA) are directed against the reticular fibers of endomysium, a layer of connective tissue which sheathes smooth muscle fibers. Testing for antigliadin antibodies by enzyme-linked immunosorbent assay (ELISA) was developed in the early 1980s. Although still available for diagnosis, AGA immunoassays demonstrate variable clinical performance, particularly in adults, and are not recommended in screening patients with symptoms and/or at risk for CD (4, 1, 2). Testing for ARA and/or AGA is no longer advocated for screening individuals who have CD symptoms or are at risk for CD (4, 21, 2). While testing for AGA has largely been replaced by the more-specific anti-DGP antibody assays, ARA tests are requested by quite a number of clinicians in the routine evaluation for CD.

CONCLUSION
Celiac disease is a complex, systemic disease affecting the growth, development, and quality of life of a significant proportion of the population. Detection of anti-tTG and/or EMA antibodies represent the cornerstone for identifying patients with CD and/or at risk for disease. The use of ARA testing deviates from current recommendations for serologic screening. There are very few recent clinical investigations comparing the diagnostic significance of ARA to contemporary serologic tests for CD. Based on the results from these limited studies and their performance in past investigations, their use in current practice is unwarranted. In addition, our current knowledge of CD-specific serologic testing and the immunobiology of disease lead us to conclude that ARA testing is no longer useful in the routine evaluation of both patients with CD symptoms and individuals at risk for CD.

REFERENCES