Objectives

At the end of this presentation you will be able to:

1. Discuss the basics of Celiac disease and its progression
2. Explain the complexity of symptoms of Celiac Disease
3. Review modern testing techniques
4. Describe the basis of disease control

What is Celiac Disease?
(aka Sprue, Celiac sprue, gluten-sensitive enteropathy)

- Celiac Disease is an immune-mediated intestinal disorder characterized by chronic inflammation of the small intestine mucosa triggered by the gliadin component of ingested gluten.
- Damage to the intestine can result in the total atrophy of intestinal villi, malabsorption, and a multitude of clinical manifestations.
- Treatment of Celiac Disease: 100% elimination of gluten-containing grains (wheat, rye and barley) from the individual’s diet
History

Accounts of celiac sprue date back to the first century A.D.

Willem K Dicke, a Dutch pediatrician in the 1940's first linked it to gluten when he observed that the condition of children with celiac sprue improved during the food shortages of World War II, only to return when cereal supplies were restored after the war.
Celiac Disease

- 1% of population
- Most individuals are undiagnosed
- Many symptoms
- Some no symptoms
- Both Diagnosis and monitoring difficult
- Repeat testing

Celiac disease prevalence

- ~1:100 in US and Europe. Rare in African-Caribbean, China or Japan. WHY???
- Associated with HLA-DQ2 and/or DQ8 in 95% of patients.
- Often found in relatives that may be asymptomatic.

Other Information

- 1:100 Celiac disease patients are IgA deficient; compared to 1:1,000 in population.
- Undiagnosed and untreated Celiac disease patients have higher rate of gastrointestinal cancers.
**Celiac disease in children**
- In **young children (pre-pubescent)** it can occur as early as 4-36 months or any time thereafter as grains are introduced into the diet.
- Manifests with diarrhea (or constipation), abdominal distension, abdominal pain, bloating and failure to thrive.
- With severe untreated disease, children may have short stature, delayed puberty, iron and folate deficiency with anemia, weight loss, stunted growth, and rickets.

**Celiac disease in Adolescents**
In **older children (teens)** it may present with:
- Arthralgia
- Nausea and/or vomiting
- Chronic abdominal pain, cramping or distension, chronic constipation
- Abnormal liver biochemistry
- Recurrent aphthous stomatitis (mouth ulcers), defects in dental enamel,
- Dermatitis herpetiformis – like rash

**Celiac disease in Adolescents**
- Fracture with inadequate traumas / osteopenia / osteoporosis
- Chronic fatigue
- Behavioral disturbances such as depression
- May be irritable
- May perform poorly in school.
Celiac disease in adults

Adults may present with:

- Diarrhea, although about 50% don’t have clinically significant diarrhea
- Flatulence
- Abdominal discomfort and bloating
- Weight loss
- Symptomatic lactose intolerance.
- Malaise
- Coagulopathy resulting from vitamin K deficiency

Celiac disease in adults

- Macrocytic anemia due to folate (or vitamin B) deficiency
- Vitamin D deficiency leading to hypocalcemia
- Anemia (severe during pregnancy)
- Iron-deficiency anemia is the most common presentation
- Bone fractures and osteoporosis
- Psychiatric syndromes
- Neurologic conditions, including peripheral neuropathy, ataxia, and seizures

Dietary Guidelines for Patients with Celiac

- Avoid all food containing wheat, rye, and barley gluten.
- Initially avoid all foods containing oats
- Initially avoid all foods containing lactose
- Use only rice, corn, maize, buckwheat, potato, soybean, or tapioca flours, meals or starches
Dietary Guidelines continued

- Use foods that have the gluten-free symbol
- Once under control, you can try foods containing gluten-free wheat starch
- Gluten is also contained in medications, food additives, emulsifiers, and stabilizers
- Avoid Most beers, lagers, ales, and stouts

DGP ELISA (Deamidated Gliadin Peptide)

Celiac Disease - Pathology

- Villous atrophy
- Hyperplasia of the crypts
- Increased numbers of intraepithelial lymphocytes.
- The immunogenic area of Gluten in celiac patients is a 33-amino-acid peptide that carries multiple copies of 3 epitopes.

Celiac Disease

- Normal intestinal villi create 200 sq meters of absorption area
- Total villous atrophy decreases the absorption area to about 2 meters

Biopsies

- Until the last few decades, biopsy was the only way to diagnose Celiac Disease
- Biopsies should be taken during upper endoscopy from the bulb (at least 1 biopsy) and from the second or third portion of duodenum (at least 4 biopsies).
- The alterations are patchy and not specific for CD, these may be found in enteropathies other than CD.
Biopsy Problems

Other causes of villous atrophy in duodenum
- Tropical sprue
- Small-bowel bacterial overgrowth
- Autoimmune enteropathy
- Hypogammaglobulinemic sprue
- Drug-associated enteropathy (e.g., olmesartan)
- Whipple disease
- Collagenous sprue
- Crohn’s disease
- Eosinophilic enteritis
- Intestinal lymphoma
- Intestinal tuberculosis
- Infectious enteritis (e.g., giardiasis)
- Graft versus host disease
- Acquired immune deficiency syndrome enteropathy
- Malnutrition

Marsh Stage of Celiac Disease

- **Stage 0**: The mucosa (intestinal lining) is normal. No lymphocytic infiltration.
- **Stage 1**: The cells on the surface of the intestinal lining (the epithelial cells) are being infiltrated by lymphocytes.
- **Stage 2**: The changes of Stage 1 are present (increased lymphocytes), and the crypts (tube-like depressions in the intestinal lining around the villi) are "hyperplastic" (larger than normal).
- **Stage 3**: The changes of Stage 2 are present (increased lymphocytes and hyperplastic crypts), and the villi are shrinking and flattening (atrophy). There are three subsets of Stage 3:
  - Partial villous atrophy (Stage 3a)
  - Subtotal villous atrophy (Stage 3b)
  - Total villous atrophy (Stage 3c).

Dermatitis Herpetiformis

- A pruritic blistering disease.
- Classically occurs in 2nd-3rd decade.
- Erythematous papules and/or vesicles localized over the extensor surfaces.
- Most are symmetrically distributed on elbows, knees, buttocks, back and posterior hairline.
Dermatitis herpetiformis
Cutaneous manifestations

Laboratory Testing for Celiac Disease
- Serologic tests for celiac disease
  - Reticulin IgA
  - ELISA – Gliadin IgA and IgG
  - Endomysial antibodies, IgA
  - Tissue transglutaminase antibodies, IgA & IgG
  - Deamidated gliadin antibodies, IgA and IgG
  - F-actin IgA
- HLA typing for celiac disease
  - HLA-DQ2 and HLA-DQ8
Reticulin Antibodies

- Substrate is rat or mouse Kidney+Stomach.
- Tested using anti-IgA conjugate.
- Serum diluted 1:20.
- Assay is not used currently due to poor sensitivity and specificity.

Reticulin Fibers

Gliadin antibodies- IgA, IgG

1. Tested by ELISA
2. Native Gliadin assays have fair sensitivity, but low specificity for Celiac Disease
3. Native Gliadin IgG antibodies are good markers for non-celiac gluten enteropathies (autism, neurologic and other problems)
Native Gliadin vs Deamidated Gliadin Peptide and tTG antibody tests

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<thead>
<tr>
<th></th>
<th>Native Gliadin IgA</th>
<th>Deamidated IgA</th>
<th>tTG IgA</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>52%</td>
<td>91%</td>
<td>89%</td>
</tr>
<tr>
<td>Specificity</td>
<td>46%</td>
<td>98%</td>
<td>98%</td>
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Endomysial antibodies

1. Substrate is monkey esophagus.
2. Tested using anti-IgA conjugate.
3. Serum diluted 1:2.5, 1:5 or 1:10.
4. Has been considered to be the "gold standard"?
5. Results vary between labs due to subjectivity and standard variability of IFA.
tTG antibodies Tested by ELISA or Chemilluminescence

- Antigens include guinea pig liver tTG, recombinant human tTG, and purified human h-tTG.
- The tTG guinea pig assay is more sensitive but less specific than the EMA.
- The recombinant anti human tTG ELISA assay is as sensitive and slightly more specific than guinea pig tTG.
- The native human tTG from human RBC is generally more sensitive and more specific than recombinant or guinea pig tTG.
Serum Hemolysis

- H-tTG is extracted from RBC’s
- It has been shown that hemolyzed samples lower EMA and/or tTG results or cause them to be falsely Negative
- Hemolyzed samples have little or no effect on the DGP assay results

Practical Observation: Celiac Testing with tTG ELISAs

HEMOLYSIS INTERFERES WITH THE DETECTION OF ANTI-tTG ANTIBODIES IN CELIAC DISEASE
Arguelles-Grande, Norman, Bhagat, Green

Hemolysis and tTG Assays Conclusion

- Hemolysis can interfere with diagnosis of celiac disease using tTG IgA assays
- Affects both red cell and recombinant-based tTG IgA assays
- Sequestration of tTG antibodies by tTG antigen released in hemolyzed sera can reduce measured titers of tTG antibodies
- Decrease of tTG titer with increased amount of hemolyzed blood
Development of deamidated gliadin peptide (DGP)

- Gliadin antibodies in celiac patients bind to a limited number of specific epitopes on the gliadin molecule.
- The body breaks down the gliadin molecule and the tTG enzymes deamidate these particles (converts glutamines to glutamic acid = water soluble)
- Deamidation of these specific gliadin molecule chains by tTG or other means enhances reactivity.
- IgA antibodies to DGPs were demonstrated to be both more sensitive and specific than IgA antibodies to native gliadin.

Celiac assays with Children

1. Children <2 to 4 with Celiac Disease are negative for tTG antibodies
2. These children are generally positive for DGP antibodies
3. If both DGP and tTG antibodies are present, a biopsy may not need to be done
4. DGP IgA or h-tTG IgA antibodies if present, can be used to monitor patients for compliance of a gluten-free diet.

F-actin IgA in Celiac Disease

- It has correlation with the degree of villous atrophy.
- Associated with a more severe degree of intestinal villous atrophy.

Testing for IgA f-actin antibodies:
- Will Decrease need for endoscopy ?
- Be an additional marker to support diagnosis?
F-actin IgA in Celiac Disease

- Correlation with degree of villous atrophy
- May decrease need for endoscopy
  - May have value for monitoring
  - Additional marker to aid in diagnosis
  - Assay useful for assessing severe intestinal damage without need for invasive procedures
  - FDA-cleared ELISA

Correlation of F-actin IgA Antibodies with Mucosal Histopathology

- 89.2% f-actin IgA+ = Marsh 3a,b,c
- 59.4% of those with most severe intestinal damage were f-actin IgA positive
- 70% of 86 f-actin IgA+ = Marsh 3c

HLA Typing

- Not used for general population screening
- HLA typing is used for familial studies or symptomatic patients with negative serologic tests.
- Should not be used for screening of symptomatic patients with positive serologies.
Dual Positive or Dual Negative Results

- Dual positive or dual negative results of tTG, EMA, DGP are highly accurate for detecting celiac disease with high clinical confidence
- Triple positive or negative results are even better!
- Increased clinical confidence reduce reliance on biopsy as required “gold standard”

ESPGHAN & NASPGHAN
Statement 2013

- DGP &/or tTG may be used to diagnose Celiac without a biopsy if the serologic values are >10 times the normal cut off.
- Children ≤ 2 or early onset adults may be positive for DGP only.
- Native gliadin antibody tests should NOT be used to test for Celiac Disease, only used for non-celiac gluten enteropathy testing.

Is Your Mind Going in Circles Now?