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Review Article

Celiac disease and its histopathology

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ABSTRACT

Celiac disease is gluten induced enteropathy and is a chronic inflammatory disorder of the small intestine characterized by malabsorption. It is a common immune mediated disorder which is triggered by consumption of wheat (gluten). It occurs in genetically predisposed individuals (carriers of HLA-DQ2 and DQ8 haplotypes). It is characterized by inflammation of the small-intestinal mucosa and myriad gastrointestinal and systemic manifestations. A duodenal biopsy with positive serology is the gold standard for the diagnosis of Celiac disease. As there are changing presentation for Celiac disease, communication of pathologist and gastroenterologists is essential for appropriate interpretation of duodenal biopsy.

INTRODUCTION

Celiac disease (CD) is a common disease, affecting 1% of the population and evidence suggests that prevalence is increasing.[1] CD is known as gluten-sensitive enteropathy, celiac sprue or nontropical sprue. It is a chronic inflammatory disorder of the small intestine characterised by malabsorption after ingestion of wheat gluten or related derivatives of barley and rye in individuals with a certain background. The pathogenesis involves a T cell mediated immune response and autoreactive B lymphocytes that produce autoantibodies directed against gliadin, endomysium or tissue transglutaminase in individuals with a genetic susceptibility related to HLA- DQ2 and HLA-DQ8.[2,3]

The clinical manifestations of CD are changeable in nature and vary markedly with the age of the patient, the duration and extent of disease and the presence of extra intestinal pathological conditions. In addition to classical gastrointestinal form, a variety of other clinical manifestations of the disease has been described including atypical and asymptomatic forms.[4]

Diagnosis of CD is extremely challenging. Serological tests developed in the last two decades provide a non invasive tool to screen both individuals at risk for the disease and the general population. The diagnosis is based on the

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Table 1 : The modified Marsh classification of gluten induced small intestinal damage

Stage 0	Preinfiltrative mucosa, up to 30% of patients with dermatitis herpetiformis (DH) or gluten ataxia have small-intestinal biopsy specimens that appear normal (fig.1)
Stage 1	Increase in the number of intraepithelial lymphocytes (IELs) to more than 30 per 100 enterocytes (fig. 2)
Stage 2	Crypt hyperplasia. In addition to the increased IELs, there is an increase in crypt depth without a reduction in villus height. Gluten challenge can induce these changes, which can also be seen in 20% of untreated patients with dermatitis herpetiformis and celiac disease
Stage 3	Villous atrophy: A- partial (fig. 3), B- subtotal, C- total (fig.4). This is the classic celiac lesion and is found in 40% of DH patients. Despite marked mucosal changes, many individuals are asymptomatic and therefore classified as having subclinical or silent cases. This lesion is characteristic of, but not diagnostic of, celiac disease and can also be seen with severe giardiasis, infantile food sensitivities, graft versus-host disease, chronic ischemia of the small intestine, tropical sprue, immunoglobulin deficiencies, and other immune deficiencies and allograft rejection

(Ref: Celiac disease, World Gastroenterology Organization Global Guidelines, April 2012)

detection of specific auto antibodies (anti- transglutaminase type 2 IgA) and compatible findings at duodenal histology like surface enterocyte damage, increased intraepithelial lymphocytes (IELs), crypt hyperplasia and villous atrophy being considered the principal hallmark.[2,4,5]

The disease should be detected as early as possible because untreated CD is associated with many severe complications such as intestinal lymphoma, cancer and osteoporosis.[4]

HISTORY

Celiac disease came into accounts as early as 1st century AD when the physician Celsus introduced the Latin term "celiac" to indicate a diarrhea like disease. Later in 250 AD and the clinical signs were described by Areteo Cappadocia. However it was Gee, who in 1888 introduced the clinical findings associated with CD in both adults and children.3,6 Gee was unable to derive an explanation for its pathogenesis and the gross morphology of the small bowel. Also the small bowel biopsy was restricted to autopsy investigations and thus the early attempts to study these tissues were hampered by autolysis.[3]

Paulley et al provided the first histopathological correlation with Celiac disease. Other important contributions were Beneke (1910), Justi (1913) and Manson-Bahr, who recognized the presence of inflammation and villous atrophy in the small intestine with Celiac disease.[3]

PATHOGENESIS

Celiac disease is widely regarded as an autoimmune disease that arises from an aberrant immune response towards derivatives of gluten, which is present in wheat, barley and rye, in genetically susceptible people.[3,7,8] Other cereals such as rice and millet are considered to be safer, as their proteins bear even less similarity to those of wheat, rye and barley.[3]

Patients with CD have a predominance of HLA Class II DQ2 and/or DQ8 molecules.[9] An individual not carrying DQ2 and DQ8 alleles is extremely unlikely to develop CD.10 Gliadin derived peptides are processed by HLA Class II molecules for presentation to helper T cells in susceptible mucosa that has perhaps been primed by a triggering effect, Helper T cells are activated and there is invasion of the surface epithelial cell by CD8 T cells. It is also proposed that there is direct gliadin toxicity on enterocytes stimulating the HLA molecules. Transglutaminase, (normal gut enzyme) which is released during injury link with gliadin forms a neopeptide. This becomes the target of an antibody response. Thus CD represents a complex array of cellular and humoral immune response.[9]

Transglutaminase has recently been identified as the epitope recognized by the antiendomysial antibody, a sensitive and specific marker of Celiac disease. Elimination of gliadin stops the direct mucosal injury and eliminates the substrate necessary to form the neopeptide that propagates immunologically mediated damage.[9,10] A diverse population of immune mediators contribute to CD including macrophages, plasma cells, CD4+T helper cells, CD8+ cytotoxic T cells and natural killer cells.[3] The inflammatory cascade produces inflammatory cytokine, proteinases and other tissue damaging mediators which damages the mucosa leading to characteristic histopathological findings.[10]

CLINICAL SPECTRUM

Celiac disease occurs both in adults and children with a female predominance (female to male ratio, 2-3:1).[10,11] The prevalence ranges from 10% to 13% in first degree relatives and a high rate of concordance (70 to 75%) in monozygotic twins compared to dizygotic twins.[3,6,10,12] The incidence is higher in wheat eating populations such as Western Europe and North America while the incidence continues to rise in Eastern societies, possibly as a result of western style eating habits.[2]

Clinical presentation varies from full blown malabsorption with weight loss, diarrhea and steatorrhea to more subtle symptoms such as folate or iron deficiency anemia, flatulence, episodic diarrhea, loose stools, neurological problems, osteoporosis and vitamin K and D deficiencies in as many as 50% of patients. Delayed puberty, infertility, protein deficiencies and elevated liver enzyme levels are also seen.[2,10,13] Celiac disease is also found to be associated with diabetes mellitus type 1. They also have increased risk

Table 2: Factors for reliable histological diagnosis
Number of biopsy procured
Quality of biopsy samples
Handling of samples
Patchiness of mucosal damage
Different grades of lesion
Subjective histologic interpretation

(Ref: Celiac disease, World Gastroenterology Organization Global Guidelines, April 2012)

of sepsis and the risk is higher for pneumococcal related sepsis.[11] One study done in Italy has shown that children born in summer are at higher risk to develop CD than children born in other seasons.[14] In children within few months of introducing the child to wheat based foods, the classic syndrome of chronic diarrhea, abdominal distension and failure to thrive appears between 6 months and 2 years of age affecting their weight and growth. In some it remains undiagnosed until adulthood.[2,15]

Celiac disease with atypical symptoms is characterized by few or no gastrointestinal manifestations and its recognition is partly responsible for the increased prevalence. Silent CD is associated with asymptomatic individuals but have a positive serologic test and typical histopathological changes. These patients are usually detected via screening of high risk individuals. Latent CD is defined by a positive serologic result but lack of symptoms and villous atrophy on biopsy but later may developed symptoms and histopathological changes.[10]

Prompt diagnosis and treatment of CD not only eases symptoms and improves quality of life but also has the potential to decrease long term risks for lymphoma, gastrointestinal carcinoma, dermatitis herpetiformis, osteopathy, endocrine abnormalities and other autoimmune disorders.[2] The overall risk of cancer is almost twice in patients with CD compared to the general population. Adherence to a gluten free diet is thought to reduce the risk of lymphoma.[10]

DIAGNOSIS

Guidelines on CD diagnosis have been published by gastrointestinal organizations since 2012. These guidelines include the combined use of biopsy and serologic analyses for diagnosis. According to American College of Gastroenterology (ACG) 2013 CD guidelines combination of both small intestinal biopsy and serologic tests (anti tissue Transglutaminase (tTG) or anti- deamidated gliadin peptide (DGP) are recommended for diagnosis of CD.[15] Guideline by European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in 2012 proposed a non invasive method of diagnosing CD in pediatric patients. These patients with symptoms consistent with CD can be diagnosed without biopsy confirmation if they have an IgA tTG titre> 10 fold above the upper limit of normal, a positive endomysial antibody (EMA) in a separate blood sample and carry the HLA DQ2 or DQ8 haplotype.[16] The British Society of Gastroenterology recommendations for adult CD suggest that serologic tests either tTG, EMA or DGP should be done followed by small intestinal biopsy for a definitive diagnosis.[1] Recent guidelines from the World Gastroenterologhical Association recommend serologic tests including anti- tTG and / or anti- EMA or anti- DGP for diagnosis and biopsy suggested but not considered mandatory for CD diagnosis.[1,17]

The gold standard of diagnosis is the small bowel mucosal biopsy together with positive serology. In 1992, Marsh reviewed the intensity of mucosal damage observed in treated CD patients who were confronted with increased amounts of gluten. A modified Marsh classification is now widely used in diagnosing CD in clinical practice.[17,18]

SMALL INTESTINAL BIOPSY

Histologic damage is considered characteristics but not pathognomonic of CD as similar lesions are seen in several other disorders. CD affects the mucosa of the proximal small intestine and less to the distal small intestine. The severity and extent of the histological damage appear to correlate with the intensity of the clinical symptoms. At least four biopsy samples must be taken, three from the second part of the duodenum distal to the papilla and one from the duodenal bulb. A second biopsy can be done in selected patients who have positive autoantibodies such as EMA.[17]

The characteristic histopathological findings seen are:

Table 3: Conditions with increased IEL and/or villous atrophy and crypt hyperplasia that can mimic Celiac disease	
Helicobacter pylori infection	↑ IEL
Drugs	↑ IEL + villous atrophy
Tropical sprue	Villous atrophy + crypt hyperplasia
Giardia lamblia infection	Villous atrophy +/-
Other infections (bacterial, parasitic)	↑ IEL +/- villous atrophy
Food allergies (eg- Cow's milk protein)	↑ IEL +/- villous atrophy
Autoimmune enteropathy	↑ IEL + villous atrophy+/- crypt hyperplasia
Inflammatory bowel disease	↑ IEL + villous atrophy

(Ref: Diagnosing Celiac disease: Role of the Pathologist, Int J Celiac disease, 2014)

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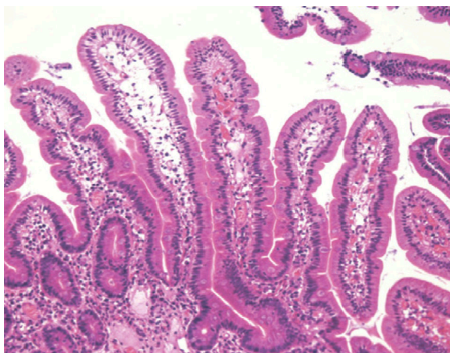

Figure 1: *Normal duodenal mucosa (villous crypt ratio: 3:1 and IELs within normal range; HE stain x100).*

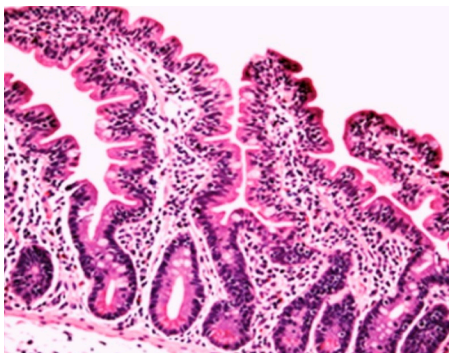

Figure 2: *Increased numbers of IELs (HE stain x100).*

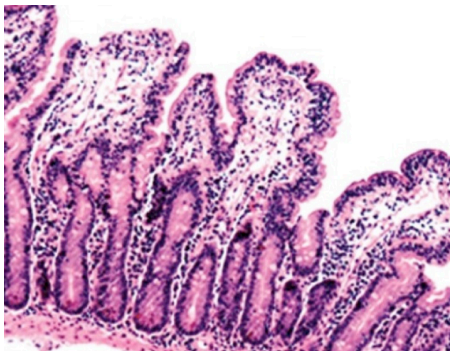

Figure 3: *Partial villous atrophy and diffuse increase in IELs(HE stain x100).*

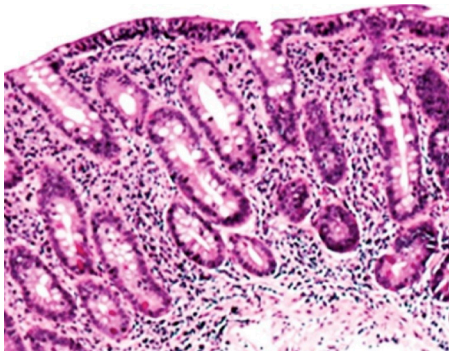

Figure 4: *Total villous atrophy and diffuse increase in IELs (HE stain x100).*

blunted or atrophic villi, crypt hyperplasia, mono nuclear cell infiltration in the lamina propria, epithelial changes, including structural abnormalities in epithelial cells and intraepithelial lymphocyte (IEL) infiltration.10,17 Increased in IEL is the first and most sensitive marker of the effects of gluten on the small bowel mucosa and is therefore the major histological feature of Celiac disease. It is suggested that a clustering of lymphocytes (>/=12) in the epithelium at the tips of villi and extending evenly down along the sides of the villus are a clue that CD may be present. Increased cellularity in the lamina propria is another important histological finding of Celiac disease. Plasma cells, lymphocytes and eosinophils are increased in number, particularly in the upper half of the mucosa. Enterocyte damage resulting in vacuolated cytoplasm is seen in severe injury. Other features of sever injury like villous atrophy and crypt hyperplasia can only be assessed in well oriented sections. Villous crypt ratios can be assessed in four or more crypts in parallel. Villi overlying and adjacent to lymphoid nodules/ follicles are normally blunted or absent and such areas should not be chosen for analysis.9

The modified Marsh classification of gluten induced small intestinal damage helps to interpret the histopathological findings ranging from normal mucosa to completely flat villi. (Table- 1)

A correct histopathological diagnosis requires factor related to number of samples, sample quality, processing and reading. There are factors to be considered for ensuring reliable histological diagnosis. (Table 2)

However there are many other conditions with increased IEL and/or villous atrophy and crypt hyperplasia that can mimic Celiac disease.18 (Table 3)

A number of serological markers have been shown repeatedly in many studies to be highly sensitive and specific for untreated Celiac disease. There are two groups of serological tests, autoantibodies- EMA, tTG antibody and antibodies targeting the offending agent (AGAs) which is now considered obsolete for diagnostic purposes because of their lower sensitivity and specificity and antibodies against synthetic deamidated gliadin peptides (DGPs). These antibodies are based on immunoglobulin A (IgA) or immunoglobulin G (IgG). IgG based tests are useful for detecting CD in IgA deficient patients.17

IgA EMA test is moderately sensitive (80%) and highly specific (almost 100%) for untreated (active) Celiac disease. Anti tTG antibodies are highly sensitive and specific for the diagnosis of Celiac disease. Deamidated gliadin peptides antibodies were introduced a few years ago and recently two DGP tests are combined in a single assay including IgA

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and IgG tTG determination.[17] Sensitivity and specificity differs from patient to patient in Celiac disease. Therefore choosing the most appropriate serologic test in different clinical scenarios is a wise thing. For confirmation of gluten dependence in patients with enteropathy, IgA EMA, IgA tTG and IgG and IgA DGP gives the good result. IgG DGP is helpful in IgA deficient patients and for some EMA-negative and tTG negative patients.[17] Serologic testing is very useful for screening patients with suspected CD as early detection is essential to prevent the complications of Celiac disease.[19]

After the diagnosis, patients should be advised for the importance of strict adherence to the diet. Serological screening of first degree and second degree relatives should be considered. Persistence of symptoms is almost always caused by continued ingestion of gluten.[17]

Refractory CD is diagnosed when symptoms persist and when there is villous atrophy and failure to respond to a gluten free diet. This may be primary (occurring at the time of presentation) or secondary (after an initial response to a gluten free diet). It is usually diagnosed after the age of 50.[17] Possible causes of refractory CD are unrecognized intake of gluten, lack of adherence to a gluten free diet and development of lymphoma. Possible findings in histology is the thickened subepithelial collagen layer, mucosal thinning and subcryptal mononuclear inflammation and evidence of lymphoma.[9] There are two subtypes of refractory CD, type I with normal IEL and type II with clonal expansion of IEL and an aberrant phenotype lacking CD3, CD8 and T cell receptors. Type II refractory CD is the most sever form and is considered to be a form of low grade intraepithelial lymphoma.[17]

When screening small bowel biopsy several features have to be considered before giving the diagnosis.[20] Hence, checklist based, templated pathology report can be beneficial to ensure capturing and reporting all relevant histopathological features.[10]

Reports should include:

• Site and number of biopsy specimens with a comment on specimen orientation.

• Villous to crypt ratio: normal (3:1 to 5:1) or abnormal

• Presence and degree of villous atrophy: normal or atrophic-mild (partial), Moderate (subtotal) or severe (total)

• Increase in IEL counts with use of immunohistochemistry for CD3 in equivocal cases.

o Normal: Fewer than 25IELs /100 enterocytes

o Borderline increased: 25-29 IELs/100 enterocytes

o Definitely increased: at least 30 IELs /100 enterocytes

• Presence / absence of surface epithelium damage

• Presence / absence of subepithelial collagen

• Lamina propria inflammation: type and degree

• Other: Clinical information and serology results, descriptive diagnosis including differential diagnosis and histopathological impression consistent with or suggestive of Celiac disease.

CONCLUSION

Celiac disease is a chronic systemic immune mediated disorder associated with variable small intestinal mucosal injury triggered by gluten in genetically predisposed individuals. Small bowel biopsy remains an essential component to the screening and diagnosis of celiac disease. With improved sensitivity and specificity of serologic testing and growing awareness among the clinicians, the diagnosis of CD is becoming easier and accurate. However, because of varied clinicopathological spectrum of the disease and many entities in the differential diagnosis of gluten sensitive enteropathy, diagnosis depends on good clinicopathological communication. The pathology report should be brief, descriptive and summary of the salient histopathological findings which can be easily assimilated by clinician. Serological testing or re biopsy can be recommended if indicated to promote standardisation and consensus among pathologists and clinicians.

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