Original Research Article

Celiac disease and its histopathology

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Abstract

Introduction: Celiac disease, also known as celiac sprue, non-tropical sprue, gluten-induced enteropathy, or gluten-sensitive enteropathy (GSE), is a chronic immune-mediated disorder of the small intestine characterized by malabsorption after ingestion of wheat gluten or related proteins in rye (secalins) and barley (hordeins) in individuals with a certain genetic background. **Materials and Method:** The study was conducted in Mahatma Gandhi Medical College and hospital, Jaipur. 53 Duodenal biopsies were taken who have symptoms of diarrhea, iron deficiency anemia, amenorrhea, recurrent mouth ulceration. All biopsies taken from duodenal sitewere brought in 10% buffered formalin. After overnight fixation in formalin, the tissues were processed in automated tissue processor for dehydration, clearing, and paraffin embedding. Sections were cut in rotary microtome 4 micrometer thickness. The section was stained for Hematoxylin and Eosin stain. **Results:** The present study included 53 Duodenal biopsies. Out of 53 duodenal biopsies, 12 biopsies have shown presence of Celiac disease. Rest 41 biopsies have shown chronic duodenitis. **Conclusion:** Celiac disease is a common autoimmune condition with mainly intestinal, but also extra-intestinal manifestations. The histologic hallmark of GlutenSensitive Enteropathy is both increased inflammation and architectural derangement in the small intestinal villi.The small intestinal mucosa of untreated persons with celiac disease usually display villous atrophy (Marsh III), or, less commonly, isolated crypt hyperplasia (Marsh II), together with intraepithelial lymphocytic proliferation.

Keywords: Celiac disease; Duodenal biopsy; Gluten sensitivity; Villous atrophy;Intraepithelial Lymphocytosis; Crypt hyperplasia

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Introduction

Celiac disease, also known as celiac sprue, non-tropicalsprue, gluten-induced enteropathy, or gluten-sensitive enteropathy (GSE), is a chronic immune-mediated disorder of the small intestine characterized by malabsorption afteringestion of wheat gluten or related proteins in rye (secalins) and barley (hordeins) in individuals with acertain genetic background[1].

The pathogenesis involves aT-cell–mediated immune response and autoreactive Blymphocytes that produce autoantibodies directed against gliadin, endomysium, or tissue transglutaminase in individuals with a genetic susceptibility related to humanleukocyte antigens HLA-DQ2 and HLA-DQ8 [1]. Celiac disease is a chronic, immune-mediated disease occurring in genetically predisposed individuals due to an intolerance to gluten-containing foods and, in particular, to some of its proteins, called gliadins. This intolerance leads to abnormal immune response, which is followed by a chronic inflammation of the small intestinal mucosa with progressive disappearance of intestinal villi [2].

The proximal small intestine is the major site of disease. Increased intraepithelial lymphocytosis, with or without concomitant villous atrophy, is the characteristic histologic finding.

While histologic examination remains the "gold standard" for diagnosis of celiac disease, changes can be subtle when duodenal villous architecture is intactand villous atrophy may also be encountered in various other conditions. In practice, a combination of clinical suspicion, morphologic abnormality, and positive serologic findings are used for the initial diagnosis of most patients with celiac disease [3].

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The symptoms associated with Celiac disease are diarrhea, abdominal distension, and failure to thrive in the setting of villous atrophy. Sometimes It is associated with extra intestinal manifestations, such as iron deficiency anemia, osteoporosis, short stature, arthritis, infertility, peripheral neuropathy, and even liver failure at the time of diagnosis [4].

Dermatitis herpetiformis is a cutaneous manifestation of small intestinal immune mediated enteropathy precipitated by exposure to dietarygluten. It is characterized by herpetiform clusters of pruriti-curticated papules and vesicles on the skin, especially on the elbows, buttocks and knees, and IgA deposits in the dermalpapillae. Dermatitis herpetiformis responds to a Gluten free diet[5].

Intestinal biopsy by endoscopy is always performed in second and third duodenal portion remain an essential means of confirming diagnosis of celiac disease. To retain its diagnostic validity, it is fundamental for the patient to be on a normal diet containing gluten at the time of biopsy [6].

Differential diagnosis of Celiac disease includes a variety ofdisorders that manifest villous atrophy and/or increased numbers of Intra epithelial lymphocytes. Intraepithelial lymphocytosis is acharacteristic histologic feature of Celiac disease; however, it is arather nonspecific finding[7].

Conditions with increased Intraepithelial lymphocyte (IEL) and/or villous atrophy and crypt hyperplasia that can mimic Celiac diseaseare 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 (e.g.- Cow's milk protein) (↑ IEL +/- villous atrophy), Autoimmune enteropathy (↑ IEL + villous atrophy+/- crypt hyperplasia), Inflammatory bowel disease (↑ IEL + villous atrophy) [8].

The spectrum of complications of classic celiac disease [9]

- Acute global/selective malabsorption (anemia and other consequences)
- Somatic and psychosocial retardation
- Impairment of quality of life
- Infertility, miscarriage, preterm birth, low birth weigh
- Osteoporosis
- Extra intestinal manifestations, e.g., neurological (cerebellar ataxia, peripheral neuropathy), renal (IgA nephropathy), pulmonary (pulmonary hemosiderosis).
- Autoimmune diseases (type 1 diabetes, autoimmune thyroiditis)
- Cancer, particularly enteropathy-associated T-cell lymphoma (EATL)
- Increased mortality

The clinical spectrum of celiac disease is very broad, an alert clinician can diagnose it early in its course and initiate a gluten free diet, which usually exerts a protective effect against complications of malabsorption and extra intestinal involvement[9].

New treatments for classic and refractory celiac disease are currently being developed [9].

Aims and Objectives

- To describe morphologic features of duodenal biopsy of patients with celiac disease.
- To describe the presence of clinical and histologic features in Celiac disease and its mimics.

Materials and Method

Setting and type of study: The study was conducted in Mahatma Gandhi Medical College and hospital, Jaipur. 53 Duodenal biopsies were taken who have symptoms of diarrhea, iron deficiency anemia, amenorrhea, recurrent mouthulceration.

Study Sample Design: Prospective Study

All endoscopic biopsies taken from Duodenal sites are brought in 10% buffered formalin. After fixation in formalin, the tissue was processed in automated tissue processor for dehydration, clearing, and paraffin embedding.

Sections were cut in rotary microtome at 4 micrometer thickness. The section was stained for Hematoxylin and Eosin stain.

Inclusion Criteria: The study will include all biopsies which havebeen done for various chronic abdominal symptoms-abdominal pain, dyspepsia, diarrhea, and also for associated systemic manifestations like anorexia, weight loss, anemia.

Exclusion Criteria: Acute symptoms, autolyzed specimen.

Bias: Selection Bias (Cases with Diarrhea)

Results

The present study included 53 Duodenal biopsies. Out of 53 duodenal biopsies, 12 biopsies haveshown presence of Celiac disease. Rest 41 Biopsies haveshown chronic duodenitis.

Table-1: Age distribution of duodenal lesion.

Age (Years)	No of cases	%
10	5	9.43
11-20	6	11.32
21-30	15	28.30
31-40	7	13.21
41-50	12	22.64
51-60	3	5.66
61-70	3	5.66
71-80	2	3.77
Total	**53**	**~100**

Most cases belong to age group between 21 to 30 and 41 to 50. Youngest age of chronic duodenitis was 10 year and oldest age of celiac disease was 76 years.

Table-2: Gender distribution of duodenal biopsy.

Gender	N= Subjects	Percentage
Female	16	30.19%
Male	37	69.81%
Total	**53**	**100%**

Chronic duodenitis is more common in male than female. 37 cases out of 53 cases are male. So male to female ratio is 2.3:1.

Discussion

This study examined a comprehensive range of histologic features of celiac disease in duodenal biopsy specimens and associated changes in specimens taken from other gastrointestinal tract sites, in a series of 53 consecutive, serology-positive, first-time cases seen in Mahatma Gandhi Medical College. Our findings confirm previous reports that celiac disease is at least twice as commonly seen in Male patients, though the reason for this is uncertain [10]. The age range at the time of diagnosis was 10 to 76 years, with 28.30% of patients aged 21 to 30 years and 22.64% of patients aged 41 to 50 or more, confirming that presentation in later life is not uncommon[11].

Histopathology of proximal small intestinal biopsies remains the gold standard to confirm a diagnosis of celiac disease. Typically, celiac disease is characterized by the triad of histological features: [12]

1) Intraepithelial lymphocytosis (IEL>30/100 epithelial cells),

2) Lamina propria inflammation, and

3) Villous atrophy.

The diagnosis of Celiac diseaseis based on the identification of histological lesions accompanied by clinical and serological consistent data. On the basis of the presence of one or more of these elementary lesions the histopathology of Celiacdisease is subdivided into different diagnostic categories according to the Marsh classification [2].

Marsh classification [2]

Type 1 or infiltrative lesion.

1. Villi architecturally within normal morphological limits (normal villa/crypt ratio 3:1);

2. Increased number of intraepithelial lymphocytes (greater than 25–30 per 100 epithelial cells).

Type 2 or hyperplastic lesion

1. Villi architecturally within normal morphological limits (like type 1);

2. Increased number of intraepithelial lymphocytes (greater than 25–30 per 100 epithelial cells) (like type 1);

3. Hyperplasia of the glandular elements (regenerative aspect of the glandular elements highlighted by the reducedmuciferous activity and increased number of mitoses).

Type 3 or destructive lesion.

1. Varying degrees of villous atrophy associated with hyperplasia of glandular crypts;

2. Reduced surface enterocyte height, with irregular brush border and sometimes cytoplasmic vacuoles;

3. Increased number of intraepithelial lymphocytes (like type 1 and 2 lesions).

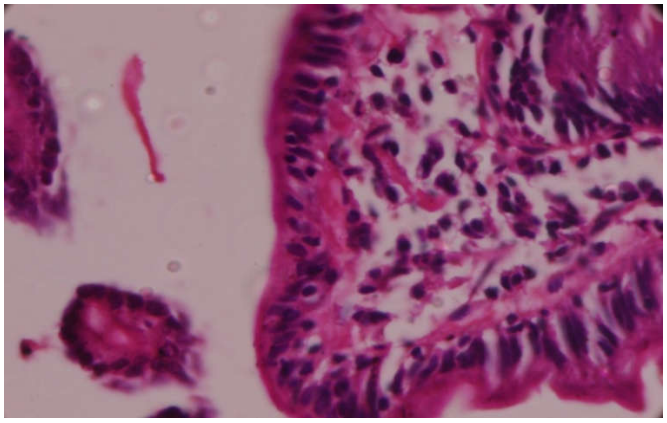

Fig 1: Intraepithelial lymphocyte in duodenal mucosa (H and E Stain 100x)

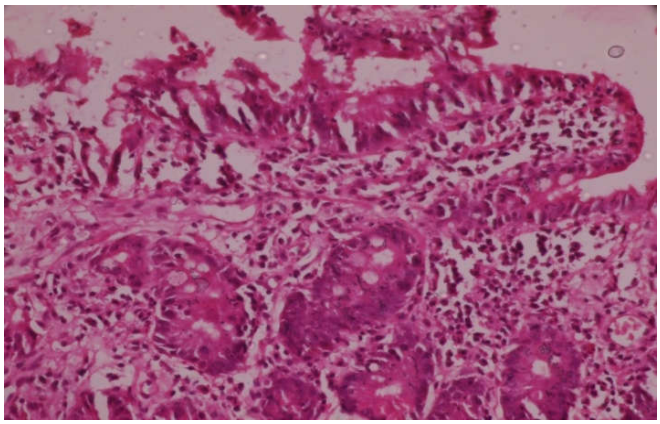

Fig 2: Villi and crypts in duodenal biopsy (H and E Stain 100x)

The most important problem today in the diagnosis ofceliac disease is represented by early lesions, i.e. normal villiwith a pathologic increase in intraepithelial T lymphocytes [13].

In addition to celiac disease, thereare a number of pathological conditions that have the samemorphological aspect as celiac disease in its early stages, i.e.normal villous architecture but with a pathological increase of IELs (>25–30/100 epithelial cells) (lesion type 1 according to Marsh, Grade A according to the new proposed classification) [2].

These conditions include hypersensitivity to other foods (milk, cereals, soybeans, fish, etc.), infections (Helicobacterpylori, Giardia, etc.), the use of drugs, immunodeficiency andimmunity dysregulation (Hashimoto thyroiditis, systemiclupus erythematosus, rheumatoid arthritis, etc.) and, not least, chronic idiopathic inflammatory bowel colitis or colitis witha different etiology, such as lymphocytic and collagenouscolitis [2]. Increased number of Intraepithelial lymphocyte and villous atrophy are not specific for Celiac disease. Increased number of Intra epithelial lymphocyte in an architecturally normal duodenal mucosa can be found in patients with Helicobacter pylori gastritis and is reduced after antibiotic treatment. Some drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) or proton pump inhibitors can cause increased Intra epitheliallymphocyte numbers, and some drugs are also capable of causing small intestinal villous atrophy (e.g. azathioprin, colchicine, ipilimumab, mycophenolate, NSAIDs, olmesartan).

Tropical sprue is an endemic malabsorption syndrome with histology similar to Celiac disease, although total villous atrophy is rare and changes are equally prominent in the jejunum and ileum in addition to the duodenum[14].

Infection by Giardia lamblia in most cases does not cause mucosal abnormalities, but variable villous atrophy can be found in a minority of cases. Patients with bacterial overgrowth often show patchy villous blunting and variable increase of chronic inflammation reminiscent of Celiac disease. Diffuse villous atrophy with increased Intraepithelial Lymphocyte can also be seen in patients with prolonged viral gastroenteritis. Other food allergies (such as cow's milk protein allergy) can be associated with increased intraepithelial lymphocyte numbersand sometimes with partial villous atrophy[14].

Autoimmune enteropathy (AIE) is a rare disease presenting with intractable diarrhea and microscopically characterized by subtotal to total villous atrophy, normal to slightly hyperplastic crypts with lymphocytic infiltration and moderate to marked chronic inflammation in the lamina propria. Increased number of Intraepithelial lymphocyte and variable degree of villous atrophy were reported in patients with various extra intestinal autoimmune disorders, including Hashimoto thyroiditis, Graves disease, rheumatoid arthritis, lupus erythematosus, multiple sclerosis, psoriasis, ankylosing spondylitis or progressive systemic sclerosis. Patients with IgA deficiency or common variable immunodeficiency (CVID) often have increased intraepithelial lymphocyte and variable villous atrophy[14].

Peptic duodenitis is characterized by edema, acute inflammation in the lamina propria and epithelium, erosions, gastric (foveolar) metaplasia and variable villous atrophy. These changes typically occur in the proximal duodenum, but rarely can be found in the distal duodenum as well. Importantly, peptic duodenitis can co-exist with Celiac disease, and can be distinguished by increased number of Intraepithelial lymphocyte.

Recent study showed that significant duodenal neutrophilia (including foci of cryptitis and crypt abscesses) is often found in patients with Celiac disease, especially in the pediatric population (56% of pediatric and 28% of adult Celiac Disease patients), and is associated with more active disease. Thus, the presence of neutrophils or foveolar metaplasia should not be used to rule-out the diagnosis of Celiac Disease [14].

Patients with reactive Crohn's disease can show only increased numbers of IEL in the architecturally normal duodenal mucosa, while in florid stage there is a variable degree of architectural distortion, active inflammation with crypt abscesses, basal lymphoplasmacytosis, pyloric metaplasia and occasional granulomas. Some patients with ulcerative colitis showed diffuse chronic duodenitis, characterized by diffuse plasmacytosis in the lamina propria, patchy cryptitis, and variable blunting of villi[14].

Complications That Can Be Confirmed Histologically[2]

- **Collagenous sprue:** The patient does not respond to diet and histology shows fibrous tissue in the intestinal wall at the level of the superficial subepithelial layer. This morphological pattern is very similar to the condition of collagenous colitis described in the colon, where the thickness of the connective band best highlighted with Masson's trichrome is more than 15 millimicrons, although this is a very rare event is described in the literature.

- **Refractory Sprue:** This condition reproduces the same clinical picture as collagenous sprue but can be identified by immuno histochemical staining, demonstrating that T lymphocytes, which in normal conditions express CD3 and CD8, in this case present only the expression of CD3 and not of CD8[15].

- **Ulcerative jejunoileitis:** Presence of extensive ulceration of the intestinal mucosa, often related to refractory sprue.

- **Lymphoma:** This is the most serious complication and should always be suspected when histology shows a prevalence of atypical mono-morphous lymphocytic elements. In these cases, it is useful to carry out immunophenotyping of the lymphoid population, which is almost always type T[16,17,18].

- Celiac disease is a common inflammatory disease of the small bowel that is precipitated by the consumption of foods that contain gluten [9].

The small intestinal mucosa of untreated persons with celiac disease usually displays villous atrophy (Marsh III), or, less commonly, isolated crypt hyperplasia (Marsh II), together with intraepithelial lymphocytic proliferation[9].

Conclusion

Celiac disease is a common autoimmune condition with mainly intestinal, but also extra-intestinal manifestations.

The histologic hallmark of Gluten Sensitive Enteropathy is both increased inflammation and architectural derangement in the small intestinal villi. The small intestinal mucosa of untreated persons with celiac disease usually display villous atrophy (Marsh III), or, less commonly, isolated crypt hyperplasia (Marsh II), together with intraepithelial lymphocytic proliferation.

Duodenal biopsy is still an essential component in the diagnosis of celiac disease.

Present study didn't use any statistical tool instead we tried to focus on histopathology of celiac disease and its mimics. In practice a combination of clinical suspicion, morphological abnormalities and positive serological findings are used for diagnosis of most patients with celiac disease.

What this study adds to existing knowledge? This study helps in an understanding of celiac disease, its early diagnosis with histopathology and treatment which helps in prevention of complications. Patients should strictly follow gluten free diet.

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Findings: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes

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