# The Value of Assessment of Intraepithelial Lymphocytes Count in Surface Epithelium of Duodenal Biopsies for the Diagnosis of Celiac Disease with the use of CD3 T-Cell Marker

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**ABSTRACT:**

**BACKGROUND:**

Coeliac disease (CD) is a chronic reversible enteropathy . Increased intraepithelial infiltrate, crypt hyperplasia, and villous atrophy are the three basic features of coeliac enteropathy.

**OBJECTIVE:**

The aim of this study was to evaluate the cutoff value number of intraepithelial lymphocyte count needed for the diagnosis of CD , this is supported by the use of CD3 T-cell immunohistochemical staining.

**PATIENT AND METHODS:**

This is a retrospective study including 25 duodenal biopsies , the cases were divided into three groups, 11 patients their biopsies show only early celiac changes supported by 9 biopsies show histological features of advanced CD, and 5 duodenal biopsies diagnosed as duodenitis.

All paraffin embedded sections were stained with H&E stain and with immunohistochemicalmarker(CD3).

**RESULTS:**

Mean age of all subjects was 19.5 years. Mean age of control group was 11.8 years. Mean age of patients with early changes was 21.8 years. Mean age of patients with celiac disease was 21.5years. There was no significant difference in mean age and sex among the three groups.

Mean IEL in celiac disease was 63.1, while it was 38.7 in patients with early changes. Mean IEL in control group was 11.6.

The least cutoff count of IEL/100enterocyte to segregate our sample into those with definite celiac disease and those who are free of disease was 20 IEL/100 enterocytes with 100 % specificity and **100%** sensitivity.

**CONCLUSION:**

Mean IEL was significantly higher in celiac disease than in patients with duodenitis and those with early changes of celiac disease

**KEYWORDS:** intraepithelial lymphocyte,duodenal biopsy,celiac disease,CD3 T-cell marker.

## INTRODUCTION:

***Definition***

Celiac disease is only one aspect of a range of possible manifestations of gluten reaction affecting the small intestine in genetically predisposed children and adults. More than 97% of those with coeliac disease will have the DQ2 and ⁄ or DQ8 markers [(1)] . In the other hand, mucosal lesions range from an architecturally normal mucosa with just an increased number of ١٠٠ intraepithelial lymphocytes (IELs), to severe villous atrophy and crypt hyperplasia' the advent of new serologic tests, including for antigliadiadin antibodies (AGA), and anti-endomysial antibodies(EMA), enabled large-scale screening studies in CD [(2)] . A life-long strict for symptomatic celiac disease patients, the introduction of a gluten-free diet (GFD)can lead to significant improvement in symptoms' Patients with complicated CD have an elevated risk for benign and malignant tumors like Malignant lymphomas , adenocarcinoma of the small bowel and oropharyngeal tumors.[(3)]

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**Clinical features and serology of coeliac disease**

The diarrhea that is characteristic of coeliac disease is (chronic) pale, voluminous and abnormally malodorous. Abdominal pain, bloating and mouth ulcers may be present, ' the changes in the bowel make it less able to absorb nutrients, minerals and the fat-soluble vitamins A, D, E, and K . iron malabsorption may cause iron deficiency anemia, and folic acid and vitamin $B_{12}$malabsorption may give rise to megaloblasticanaemia.[4] Immunoglobulin A (IgA) tissue transglutaminase (tTg) and IgA endomysial antibodies (EMA) serological tests show high levels of sensitivity and specificity in the diagnostic process [5] .

Histopathology: Increased intraepithelial T lymphocytes:a value between 25 and 29 IEL/100 enterocytes is considered border-line value; >30 IEL/100 enterocytes represents a pathological "lymphocytosis".

- Decreased enterocyte height, flattening of enterocytes,
- Crypt hyperplasia ,extension of the regenerative epithelial crypts associated with changes in the presence of more than 1 mitosis per crypt.
- Villous atrophy :decrease in villous height, alteration of normal crypt/villous ratio (3:1) until total disappearance of villi. This assessment requires proper orientation of the biopsies [6].

**Marsh classification: 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 increased number of intraepithelial lymphocytes (greater than 25–30 per 100 epithelial cells) (like type 1).
2. Hyperplasia of the glandular elements (regenerative aspect of the glandular elements highlighted by the reduced muciferous 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).



The combination of the three factors described above is consistent with a diagnosis of coeliac disease or gluten sensitive enteropathy, which should be considered as synonyms[7] .A new version of the histological classification has recently been proposed by Corazza and Villanacci [8] ,the lesions that characterize coeliac disease have been divided into two categories: Non-atrophic (grade A) and atrophic (grade B).

Grade A lesions are characterized by a pathological increase in intraepithelial lymphocytes with normal villi architecture, best recognized by the use of immunohistochemical techniques, Grade B lesions are further subdivided into:

Grade B1 in which the villus/crypt ratio is less than 3:1, with villi still identifiable and Grade B2 in which the villi are no longer identifiable

**Diagnostic criteria :** The final diagnosis in the case of an atrophic lesion, culminating in a "consistent" with a CD with atrophic lesions (type 3a, 3b or 3c); in the case of non-atrophic lesions culminating in finding attributable to intraepithelial lymphocytosis, stressing that these injuries are "suggestive for" but not exclusive of CD and should therefore necessarily be placed in the right clinical setting and supported by a serological confirmation [9].

***Intraepithelial lymphocytes*** **:**an increase in the IELs count ⁄ 100 enterocytes along villi in coeliac disease is a cardinal diagnostic feature for coeliac disease. counting IELs should be effective and time-efficient. Simply counting IELs ⁄ 20 enterocytes at the tips of five villi is a time-efficient method to assess IELs and is both sensitive and specific[10][11] .The distribution of IELs has been stated to be important but while, in architecturally normal villi, increased IELs at the villus tip are seen in gluten-sensitive enteropathy, an even distribution along the entire length of the villi is even more common in CD.[12] In biopsies with normal architecture, the distribution of CD3+ lymphocytes in a top-heavy (tip-predominant) pattern is suggestive of coeliac disease; the use of CD8 as a marker is helpful, showing a tip-predominant pattern in all coeliac cases, but also in 56% of those without coeliac disease. This is explained by the fact that IELs are mainly cytotoxic CD4+ CD8) T cell receptor

(TCR)+ cd+ T cells, usually absent in other conditions, present in a majority in coeliac disease. .[13]

## PATIENTS AND METHOD:

### PATIENT:

A retrospective study including 25 cases, grouped as frank celiac disease histologically and negative for celiac disease which serves as control and that shows early celiac changes and need to be confirmed according to pathology reports of the cases collected.

These cases where all collected from the pathology department of the gastroenterology specialized hospital in Baghdad medical city from a period between September 2013 to September 2014. The cases that show only early histological changes of CD cover the period of study. Clinical information including age, gender, clinical manifestations have been taken from patients archive files . The patients were all on gluten containing diet comparable to that of the general Iraqi population.

For each case, one representative 4 micron section was stained with Hematoxylin and Eosin and the histopathological diagnosis was revised while 4 micron sections were put on positive charged slides and stained immunohistochemically for CD3which is tumor marker ,monoclonal mouse Anti-Human CD3 class, this high specificity, combined with the presence of CD3 at all stages of T-cell development , makes it a useful immmunohistochemical marker for T-cells in tissue sectioned .

***Assessment of immunohistochemical staining***

For CD3, cells labeled by the antibody display a staining almost entirely confined to the cytoplasm and/or cell membrane.

## RESULTS:

**Comparison of sex ratio and mean age among patients groups**

There was no significant difference in male to female ratio among the three groups.

**Table 1: Number and percentage of males and females enrolled in the present study**

**There was no significant difference in mean age among the three groups. These results are shown in figure 1.**

| | Control | | Early changes | | Celiac disease | | Total | |
|---|---|---|---|---|---|---|---|---|
| Gender | No. | % | No. | % | No. | % | No. | % |
| Male | 3 | 60.00 | 4 | 36.36 | 5 | 55.56 | 12 | 48.00 |
| Female | 2 | 40.00 | 7 | 63.64 | 4 | 44.44 | 13 | 52.00 |
| Total | 5 | 100.00 | 11 | 100.00 | 9 | 100.00 | 25 | 100.00 |
| P1 =0.596 | Control vs Early changes | | | | | | | |
| P1 =1.000 | Control vs Celiac disease | | | | | | | |

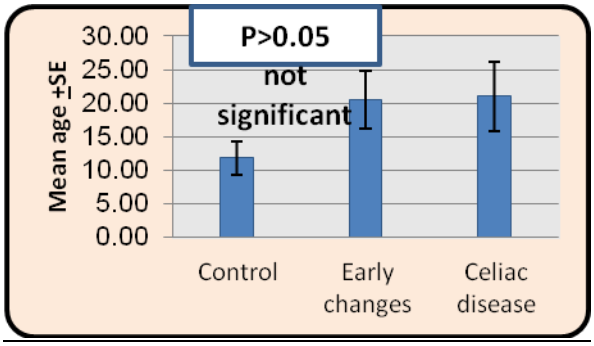



**Figure 1: Comparison of mean age among study groups.**

Comparison of intraepithelial lymphocyte mean count (IEL/100enteroctye) among study groups : Mean IEL was significantly higher in celiac disease than in control group and in patients with early changes

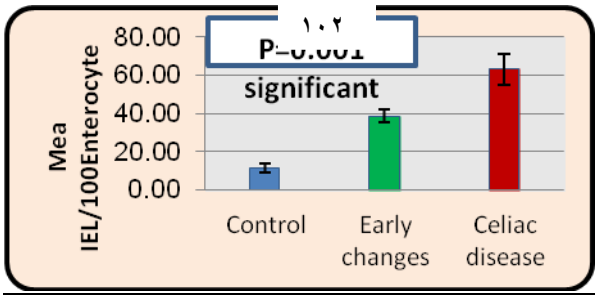



**Figure 2: Comparison of mean IEL count among study groups**

***Estimation of minimum cutoff value of IEL/100enterocyte :***

In order to fulfill aim of the present study a proper statistical tool, receiver operator characteristic (ROC), was used. This test permits finding of the least cutoff count of IEL/100enterocyte to segregate our sample into those with definite celiac disease and those who are free of disease.First step analysis we classify entire study sample into two main groups: a control group with duodentits, while the second group included patients with early changes, and the third group, patients with definite diagnosis of celiac disease, test revealed that the best cutoff value was **20** IEL/100enterocye with **100** % specificity and **100%** sensitivity. The accuracy of the test, depending on the above mentioned cutoff value was 100%. For more detailed description table **2** and figure **3** can be reviewed.

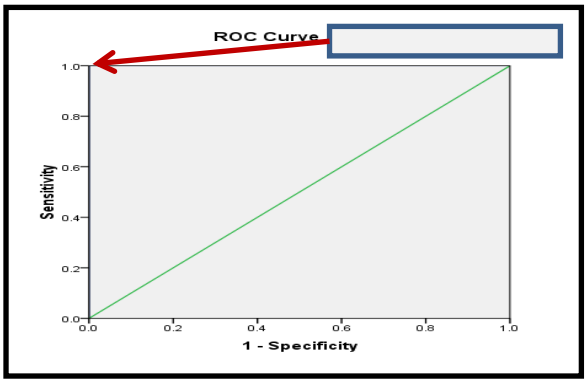



**Figure 3: Receiver operator characteristic curve showing IEL cutoff count to suggest celiac disease.**

**Table 2: Coordinates of ROC ıt suggest celiac disease.**

| | Coordinates of the Curve | |
|---|---|---|
| IEL/100 enterocyte ≥ | Sensitivity | 1 – Specificity |
| 6 | 1.000 | 1.000 |
| 8 | 1.000 | 0.800 |
| 9.5 | 1.000 | 0.600 |
| 11.5 | 1.000 | 0.400 |
| 16 | 1.000 | 0.200 |
| 20 | 1.000 | 0.000 |
| 22.5 | 0.947 | 0.000 |
| 29 | 0.895 | 0.000 |
| 34 | 0.842 | 0.000 |
| 36 | 0.789 | 0.000 |
| 38.5 | 0.737 | 0.000 |
| 41 | 0.579 | 0.000 |
| 43.5 | 0.526 | 0.000 |
| 47.5 | 0.474 | 0.000 |
| 65 | 0.211 | 0.000 |
| 81.5 | 0.158 | 0.000 |
| 84 | 0.105 | 0.000 |
| | 0.053 | 0.000 |

In the second step we classified our sample into two main groups: the first one included only patients with definite celiac disease, while the second group included the rest of the sample enrolled in the present study.

Then an ROC curve test was performed. The accuracy of the test was less than in previous step, it was 85.4%. The cutoff value was 47 IEL/100enterocyte. The sensitivity was low, 66.7% only. Specificity was acceptable, 81.2 %. For more detailed description table 3 and figure 4 can be reviewed.

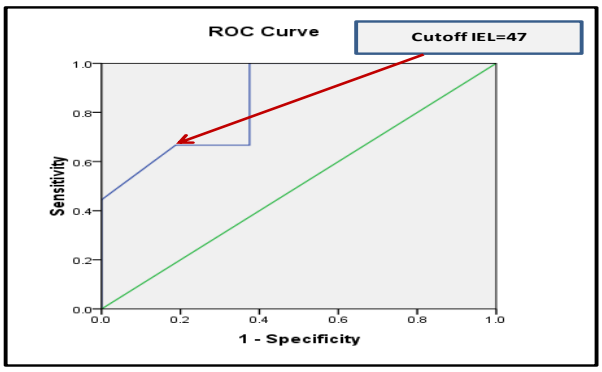



**Figure 4: Receiver operator characteristic curve showing IEL cutoff count to diagnose celiac disease.**

**Table 3: Coordinates of RO ١٠٤ hat diagnose celiac disease.**

| | Coordinates of the Curve | |
|---|---|---|
| IEL/100 enterocyte ≥ | Sensitivity | 1 – Specificity |
| 6 | 1.000 | 1.000 |
| 8 | 1.000 | 0.938 |
| 9.5 | 1.000 | 0.875 |
| 11.5 | 1.000 | 0.812 |
| 16 | 1.000 | 0.750 |
| 19.5 | 1.000 | 0.688 |
| 22.5 | 1.000 | 0.625 |
| 29 | 1.000 | 0.562 |
| 34 | 1.000 | 0.500 |
| 36 | 1.000 | 0.438 |
| 38.5 | 1.000 | 0.375 |
| 41 | 0.667 | 0.375 |
| 43.5 | 0.667 | 0.312 |
| 47 | 0.667 | 0.188 |
| 65 | 0.444 | 0.000 |
| 81.5 | 0.333 | 0.000 |
| 84 | 0.222 | 0.000 |
| 92.5 | 0.111 | 0.000 |

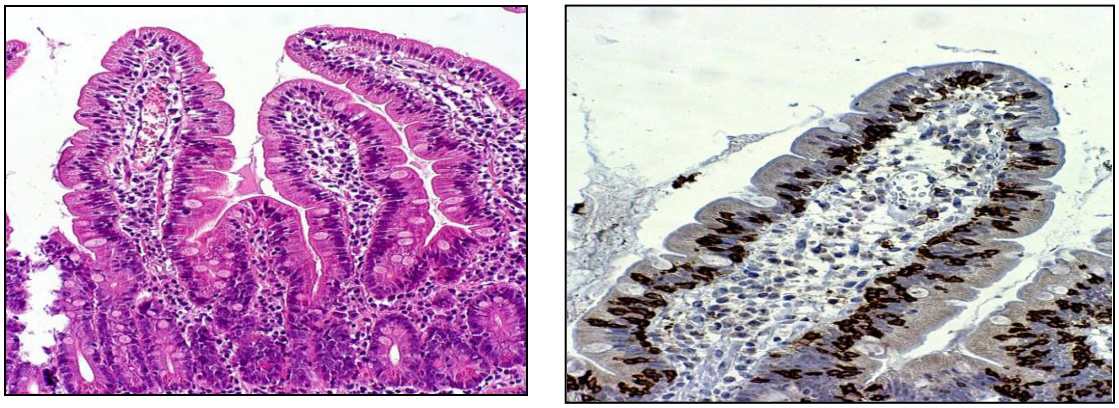
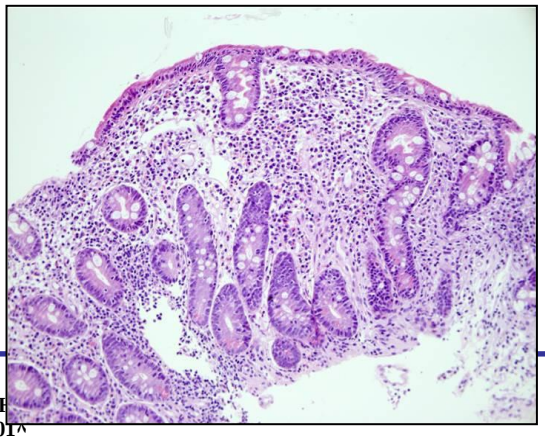


**Figure 5: A photomicrograph showing early changes of celiac disease; (A) Low power view H&E stain "10X"; (B) high power view H&E stain CD3 IHC.**



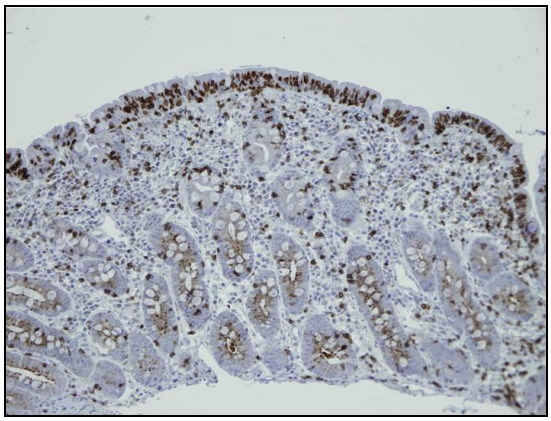


**Figure 6: A photomicrograph showing advanced celiac disease with flattened vil and prominent increase in the IEL , high power view "40X".**

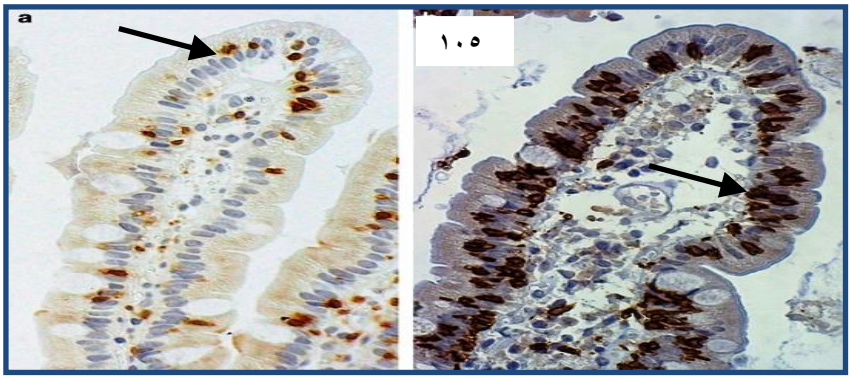



**Figure 7: A photomicrograph showing the contrast between normal (a) and increased intraepithelial lymphocyte in a duodenal villous (b). This section represents an immunohistochemical reaction with CD 3 antibodies. The power was 20X.**

## DISCUSSION:

The triad of villous atrophy, intraepithelial lymphocytosis, and chronic inflammation of the duodenum has been referred to as the 'celiac lesion. These histologic features are considered the gold standard for diagnosing celiac disease or gluten-sensitive enteropathy.[14]

An increased number of IELs is the earliest pathological sign of mild enteropathy coeliac disease.[15]

Both sensitivity and specificity of a diagnostic test lie in the possibility of applying it to a control population explicitly not affected by the disease, and a study population known to be affected by the disease. Therefore, it is crucial to determine the upper normal value of duodenal IELs in control populations in which coeliac disease has been excluded with certainty to avoid misleading results.[16]

In patients with an architecturally normal duodenal mucosa, the IEL count in villous tips helps to distinguish between patients with celiac disease and non-coeliac controls.[10]

This study was undertaken to determine the cutoff value of IEL that differentiate normal duodenal biopsy from that of potential celiac disease with normal villous pattern (Marsh 1 according to the grading system).

Regarding the **gender** , In this study there was no significant difference in male and female ratio in each of group selected, while celiac disease as with many other autoimmune diseases, is more common in female as shown by different studies.[17] with a female to male ratio of between 2:1 and 3:1By contrast, patients over the age of 60 who are diagnosed as having celiac disease are more frequently male.[18]and other recent study in India by Nijhawan et la in 2013 show that prevalence seems to favor males.[19]

The nearly equal male to female ratio in our study may be due to limited numbers of cases

selected in which the number of patients with potential celiac disease, whom the study based on, are already few.

Regarding the age there was no significant differences in the age among the all selected patients weather they show microscopic celiac changes or not, as Celiac disease can be diagnosed at any age, with a peak at early childhood and at the fourth and fifth decade of life for women and men, respectively.[20]other study in 2010 by Dennise Maan and reviewed by Laura J Martin show that celiac disease can develop at any age.[21]

Regarding the mean **value** of IEL in normal (control) group which was 11.6 in our study which is compatible with the normal range of distal duodenal IEL in which the upper limit is 20 IEL/100 enterocytes as shown in a workshop by Hayat et la in 2002 and Veress in 2000. [12] and other study in digestive center in Tehran in 2008 which also consider IEL below 34/100 enterocytes is normal. [22]

Although other studies show differences in the normal range of duodenal IEL Most of the discrepancy seems to be due to the different method used, different sample sizes, and probably different populations studied as clarified by an Iranian workshop by S. Nasseri-Moghaddam et al.in 2008.[22]

Regarding the mean value of IEL in patients that show early changes of celiac disease (potential celiac disease), the value in our study was 38.7 ,

this was obviously higher than the mean value of the control and close similar with a study by F.Biaggi in pravia, Italy in 2004. [10]

And regarding duodenal biopsies that show advanced features of celiac disease, the mean value of IEL in our study was 63.1 which much higher than control and that of early celiac changes and again these results are compatible with data of the workshop done by Hayat and Ferguson. [23]

Finally the cutoff count of IEL/100enterocyte to segregate our sample into those with definite celiac disease and those who are free of disease which was 20 IEL/100enterocye with 100 % specificity and 100% sensitivity and this result was close to a similar study by Veress in Dept. of Pathology, University Hospital MAS, Malmö, Sweden in 2004[3] and agree with other workshop in Italy in 2011 which states that the threshold of duodenal IEL may be lower than the accepted value of 25 IEL/100 enterocytes because using this cutoff value could miss nearly 50% of cases.[24]

## CONCLUSION:

1. CD is far more common than previously considered and presents as a spectrum of clinical manifestations and histologic abnormalities. The health risks for untreated celiac disease appear to be greater compared with those who adhere to a gluten-free diet.
2. CD is common but underdiagnosed, and factors related to the performance of duodenal biopsy may contribute to underdiagnosis.
3. There are many individuals with undiagnosed CD in the general population and the health impact of this cannot yet be established. Duodenal biopsy examination remains the gold standard for diagnosis of CD.
4. The degree of atrophy should be certain and not merely pseudo-atrophy due to incorrect orientation and cutting of the villi. Assessment of the number of intraepithelial lymphocytes is useful in these cases and "must" always be pathological (> 25-30/100 epithelial cells), best evaluated both with H & E staining and with immunohistochemistry staining for CD3.
5. The counting of intraepithelial lymphocytes (IELs) in the villous tips of architecturally normal small bowel biopsy specimens is a very simple and sufficiently reliable method for the assessment of the possibility of CD.
6. A raised IEL count in an architecturally normal duodenal mucosa always suggests PCD to the pathologist.

## RECOMMENDATIONS



1. Duodenal biopsy is the slandered method foı the diagnosis of celiac disease.
2. Excellent quality of the biopsy sample and correct orientation of the biopsy and sufficient clinical information are mandatory for certainty in the diagnosis of celiac disease.
3. In patients with an architecturally normal duodenal mucosa, the IEL count in villous tips helps to distinguish between patients with potential coeliac disease and non-coeliac controls.

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