Normal intraepithelial lymphocyte counts in duodenal biopsies and histological features of celiac disease among Jordanian adults

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Abstract

Objective: The earliest histopathological feature of celiac disease (CD) is increased intraepithelial lymphocytes (IEL). The aim of this study is to find out the normal IEL count and to describe the histopathological features of CD among Jordanian population.

Methods: This retrospective cohort study included 207 patients, 99 with CD cases and 108 normal controls. IEL were counted in all cases and controls. Histopathological features including villous atrophy and crypt hyperplasia were evaluated in CD cases and were classified according to the Modified Marsh classification. A two-tailed t-test was used to compare the means of variables and a P value of <0.05 was considered significant. Pearson correlation was used to measure relationships between histopathological features in CD cases. The cut-off point was suggested as the mean of normal + 2 standard deviations (SD).

Results: The mean number of IEL in normal biopsies is 11.7. The upper limit of normal is 17, this figure (17) is also the mean + 2SDs. There is no statistical difference of the number of IEL between males and females in the normal group (p = 0.54) or the CD group (p = 0.807). The number of normal IEL significantly decreases with higher age of 49 (p = 0.034).

Conclusion: Using 17 as a cut-off point is sufficient to detect CD. Higher thresholds result in missing cases. The cut off points of IEL can vary among geographical areas and thresholds should take these differences into account.

Keywords: Celiac disease, intraepithelial lymphocytes, enterocytes, histopathology, duodenal mucosa.

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Duodenal intraepithelial lymphocytes.

(Fig. 1), crypt hyperplasia (Fig. 2) and villous atrophy (Fig. 3). These histological features are not specific and can be seen in other diseases such as food allergy, autoimmune enteropathy, bacterial and parasitic infections, *Giardia* spp., common variable immunodeficiency, non-steroidal anti-inflammatory drugs, and *Helicobacter pylori* infection.

Increased intraepithelial lymphocytes is an important histological feature of CD and is the earliest observed abnormality. In normal biopsies IEL are counted at the tips of villi and reported as the number of IEL per 100 epithelial cells (enterocytes). The upper limit of normal IEL was originally cited as 40/100 enterocytes however, this figure was derived from studies on jejunal biopsies, whereas nowadays, duodenal mucosal biopsies are used to diagnose CD. Recent studies suggested to decrease this figure to 30 or 25; even lower figures were suggested; a study from Italy found that using 25 as a cut-off point could result in missing 59% of cases other studies suggested using cut off point as low as 22.8 or 20.5.

The differences in the cut-off points cited in literature could be due to geographical variations in the normal lymphocyte numbers. Few studies from the Middle East investigated the normal number of IEL in duodenal biopsies. Three studies from Iran have contradictory findings; one study reported the normal mean count of IELs/100 as 19 and suggested using 20 as the upper limit of normal whereas the other two showed higher numbers of normal IEL, one suggested using a threshold of 34 and the other reported the upper limit of normal as 37.

There are no published studies regarding the normal number of intraepithelial lymphocytes in Jordan. The main aim of this study is to investigate the normal count of intraepithelial lymphocytes among a sample of JUH (Jordan University Hospital) patients and to compare it with counts in CD in an attempt to find out a reasonable cut-off point tailored to our population. A secondary aim is to present a sample of CD cases and describe their clinicopathological characteristics among JUH patients.
Materials and Methods

Cases:
This is a retrospective cohort study with a sample of 207 cases which was conducted at JUH over a three-year period from 1/1/2015 till 31/12/2017. This study included ninety-nine patient with celiac disease and one hundred and eight healthy controls.

The celiac cases were collected from the computerized records in the histopathology department at JUH. Cases included in the study had to fulfill three criteria: clinical history of gluten intolerance, serologic evidence of auto antibodies to the enzyme tissue transglutaminase (TTG) and histological features of CD including increased intraepithelial lymphocytes, villous atrophy, and crypt hyperplasia. Cases with questionable, unconfirmed diagnosis of celiac disease were excluded. Follow up biopsies after gluten-free diet were also excluded as the histological features would’ve been modified by the diet.

The controls were also collected from the computerized histopathology records by searching for normal duodenal mucosa. The clinical history for these cases was reviewed to confirm that they didn’t have symptoms mimicking celiac disease such as diarrhea or anaemia. Only cases with no such symptoms and with a histologically normal mucosa were included.

For all 207 cases and controls, demographic and clinical data was collected including age, gender and presenting symptoms.

Histological assessment:
The haematoxylin and eosin-stained glass slides were retrieved from the Histopathology Department archives and reviewed to confirm the diagnosis of either CD or normal small bowel mucosa, count the number of IEL and evaluate the villous architecture in addition to the presence or absence of crypt hyperplasia.

IEL were counted at the tips of well oriented villi in the normal biopsies, and at the villi/or flat epithelium in the CD cases. The number of IEL was recorded as the number of lymphocytes per 100 epithelial cells (enterocytes). The count was done using Olympus BX51 microscope.

Definition: Modified Marsh classification
The histological features of CD were classified according to the modified Marsh classification as follows:[28]:
1. Grade 1: infiltrative phase where the only abnormality is an increase in IEL.
2. Grade 2: the hyperplastic phase, where there is crypt hyperplasia but no villous flattening.
3. Grade 3: the villous destruction phase where there is villous atrophy that was further classified by Oberhuber as 3a (mild, partial atrophy), 3b, (marked, subtotal atrophy) and 3c (total atrophy).[28]

Statistical analysis:
The data was presented on a Microsoft Excel sheet, version 16.12. The mean, median and standard deviation were calculated for continuous data. A two-tailed t-test was used to compare the means of variables and a P value of <0.05 was considered significant. Pearson correlation was used to measure relationships between histopathological features in CD cases.

Results

Study population
This cohort comprised of 207 cases, of which 99 were confirmed celiac disease cases and 108 were normal controls.

Of the celiac disease cases, 74 (74.4%) were females; the age range was between 3 and 72 years, the mean age was 24.3 years. Of the 99 patients who were diagnosed with CD 27% presented with abdominal pain, 40% with diarrhoea, 23% with anaemia and 10% presented with other symptoms such as vomiting, bloating, and vitamin D deficiency.

Of the normal controls 64 (59.3%) were females; the age range was between 13 and 78, the mean age was 38.6 years.

Table 1 details the demographic features of the study population.

Intraepithelial lymphocyte counts
The mean number of IEL per 100 epithelial cells was 11.7 in the normal controls, compared to 37.8 in the celiac disease cases. There is a significant statistical difference between these two means with a p value of <0.0001.

Table 2 compares the number of intraepithelial lymphocytes between the controls and the celiac disease cases.

| Table 1. Demographic features of the cases and control groups. |
|---------------------------------------------------------------|
|                                 | Celiac Disease cases | Normal controls |
| Number                        | 99                 | 108            |
| Female: male ratio            | 1:0.3              | 1:0.7          |
| Age range (years)             | 3-72               | 13-78          |
| Mean age                      | 27                 | 38.6           |
| Median age                    | 37                 | 36             |
| Standard deviation            | 16.6               | 18.1           |

| Table 2. Comparison between numbers of intraepithelial lymphocytes between cases and controls. |
|------------------------------------------------------------------------------------------------|
|                                 | Celiac | Normal |
| Mean                            | 37.8   | 11.7   |
| Median                          | 37     | 11     |
| SD                              | 8.7    | 2.7    |

P < 0.001
There was no statistical difference in the number of IEL between males and females in the control group (p = 0.54) or the celiac disease group (p = 0.807). Among the normal control group, the mean IEL was close between the age groups between 3–49 but it decreases after that (p = 0.034) (Fig. 4), this trend wasn’t observed in the CD cases (p = 0.424).

There was no significant statistical difference between the mean number of IEL in CD cases presenting with abdominal pain (37.4), anaemia (39.2) or diarrhoea (39.5).

Table 3 shows the mean number of IEL in CD cases among the different modified Marsh grades.

| Marsh classification | Number of cases | Mean IEL/100 enterocytes |
|----------------------|-----------------|-------------------------|
| 1                    | 8               | 33.7                    |
| 2                    | 6               | 34.3                    |
| 3a                   | 10              | 38.1                    |
| 3b                   | 44              | 36.1                    |
| 3c                   | 31              | 41.5                    |
| Total                | 99              | 37.8                    |

There was a significant statistical difference between IEL counts among cases with no villous atrophy (Marsh 1 and 2) and total atrophy (Marsh 3c) (p = 0.014). However, there was no significant difference between IEL counts between Marsh 1 and 2 and cases with partial atrophy (Marsh 3a) (p = 0.28) or subtotal atrophy (Marsh 3b) (p = 0.22). In addition, there was no significant statistical difference between IEL numbers among cases with Marsh 3a and 3b cases (p = 0.52) or among 3b and 3c (p = 0.38).

Pearson correlation coefficient between villous atrophy and the mean number of IEL was =0.87 which indicates a strong correlation.

Crypt hyperplasia was divided into negative (no hyperplasia), mild, moderate and severe. Of the celiac disease cases, 8 cases didn’t have crypt hyperplasia whereas 42 showed mild, 25 moderate and 23 severe hyperplasia. There was a strong correlation between degree of crypt hyperplasia and mean number of IEL with Pearson correlation coefficient =0.98 (Fig. 5).

There was a statistically significant difference of IEL counts between cases with no crypt hyperplasia and those with moderate (p = 0.064) or severe hyperplasia (p = 0.07) but not mild hyperplasia (p = 0.51). Moreover, there was a significant difference of IEL between cases with mild hyperplasia and those with moderate (0.06) or severe (p = 0.03) but not between moderate and severe (p = 0.67).

Discussion

This retrospective cohort study of 99 celiac disease cases and 108 normal controls investigates the normal numbers of IEL in duodenal mucosal biopsies among JUH patients. It also describes the histopathological features of celiac disease among patients entered in this study.

The number of IEL in normal biopsies among this study sample is between 3 and 17, mean is 11.7. Notably, there is no statistical difference between the number of intraepithelial lymphocytes between males and females in the normal group (p = 0.54) or the celiac disease group (p = 0.807). The number of normal IEL tends to decrease after the age of 49. The mean number of IEL in normal controls of 3–49 years is 12.0, but 10.7 in those 50–78 years (p = 0.034).

The mean number of IEL +2 SD is 17.1 in our sample, if this value is used as the upper limit of normal, the specificity of detecting CD will be 99%. When there is villous atrophy, diagnosing celiac disease in the right clinical setting is relatively easy. The most challenging diagnostic problem is in cases where there is no villous atrophy. Fourteen of our patients had increased intraepithelial lymphocytes with no villous atrophy. In these cases, the number of intraepithelial lymphocytes was significantly different from normal (p = 0.014). Using 17 as the upper limit of normal identifies all these cases and results in a zero false positive rate.

Increasing the cut-off point to 20 as suggested by some authors25 gives similar results to a cut-off point of 17. However, using the widely cited cut-off point of 3020 misses 14% of CD cases and using 25 as suggested by some authors8, 18, 21, 22 misses 2% of cases in our sample.
This latter cut-off point was reported to miss a higher percentage of cases (59%) 27.

Although most of the literature suggests lower cut off points than the widely used ones in histopathology practice, two studies from Iran suggested increasing the threshold to 37 27 or 34 26, however the first study had a small sample of 19 normal and 15 CD patients, and the latter documented the average number of lymphocytes in villous tips as 19 but the range of IEL count was wide (7–38) 26.

The normal counts of IEL can vary among geographical areas and a cut-off point tailored to variable populations might be needed instead of following a global guideline that doesn’t take these differences into account. Our results suggest that the threshold can be even decreased among older individuals, but this needs more studies on samples focusing on that age group.

In this study we relied on H&E stained slides to count IEL. Some studies used immunohistochemical stains mainly CD3 to count the IEL, however, in histopathological practice CD3 is not used routinely. The British Society of Gastroenterology guidelines recommend using CD3 only in equivocal cases 29, as such our methods reflect the day to day practice in histopathology labs.

Regarding the histopathological features of CD, the majority of our cases (86%) were Marsh 3. This is similar to Oliveira et al results where 73% of their cases had a Marsh grade 3 30.

In the present study, there was a strong correlation between villous atrophy and the mean number of IEL (Pearson coefficient 0.87) and between degree of crypt hyperplasia and IEL counts (Pearson correlation coefficient = 0.98). This shows that IEL counts increase as the degree of mucosal damage increases.

Limitations

This is a single institution study, but JUH is one of the largest hospitals in Jordan, moreover, Jordan is a relatively small country and geographic differences that might affect diseases’ natural history among population are minimal.

Another limitation is that the normal controls were selected from a retrospective archive of patients, however, every effort was taken to review the cases to make sure they have no gastrointestinal symptoms or diseases that might affect the results of the study.

Conclusion

IEL are an important histopathological feature in diagnosing celiac disease even in patients having no crypt hyperplasia or villous atrophy. Our results show that the upper limit of normal IEL among our population is 17 IEL per 100 enterocytes, this figure is also the mean + 2SDs. Using 17 as a cut-off point can be sufficient to detect celiac disease cases even in the mildest forms (Marsh 1).

Most histopathological labs uses 25 IEL as their cut-off point and this is the recommendation of the British Society of Gastroenterology 29, however, our results and results from other published research found this a high threshold that can miss cases of CD. Moreover, geographical differences need to be taken into account when setting a cut-off point of normal IEL.

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