INTRODUCTION

Celiac disease is the most important cause of malabsorption caused by intolerance to stored protein gluten, which is found in wheat, barley and rice. Initially, it was considered to be a paediatric disease, but it is now being diagnosed more commonly in adults and elderly population. It is defined as an exaggerated immune response to ingested gluten seen in genetically susceptible individuals. It roughly affects nearly 1% of world’s population; but according to new studies, its incidence is increasing in different geographical areas partly owing to better diagnostic tools and public awareness.

Presenting complaints are typical gastrointestinal symptoms like anemia, chronic diarrhoea, bloating, flatulence, abdominal pain, and altered bowel habits. The usual age of presentation in adults is third or fourth decade of life, but more prevalent in people above 34 years of age.

Not much of data is available about the morphological and immunohistochemical markers studies regarding the types of intestinal lesions presenting as celiac disease in adult Pakistani population. It may be due to underdiagnosis and treatment on empirical grounds as the facilities of serological testing, gastric endoscope and histopathology of intestinal biopsy are still lacking even in tertiary care centres. The exact frequency of this group of diseases in adult population of Pakistan is not known and not much of clinical data has been documented about presentation of this disease.

The rationale of this study was to emphasise the importance of diagnosis, based on European Society of Pediatric Gastroenterology, Hepatology and Nutrition criteria in adult patients undergoing endoscopic biopsy for chronic diarrhoea.

The aim of the study was to determine the histopathological features of malabsorption syndrome in adult Pakistani population suspected of celiac disease.

METHODOLOGY

This descriptive study was carried out at the Department of Histopathology, Army Medical College, National University of Sciences and Technology, Islamabad, from November 2014 to December 2015. All small intestinal mucosal biopsies of adult patients (aged above 14 years) suspected of celiac disease were included. Mucosal biopsies of patients less than 14 years of age were excluded.
as well as autolysed tissue samples were excluded from the study. Clinical data was recorded on proformas.

The biopsies were collected from the Military Hospital and Combined Military Hospital, Rawalpindi. Biopsy specimens were fixed in 10% formaline for a period of 24 hours. Following this, the biopsies underwent the routine protocol of tissue grossing, processing, paraffin embedding, and sectioning. The slides thus made were stained by Haematoxylin and Eosin; and two slides of each case was stained for immunohistochemical markers CD3 and CD20. For immunohistochemistry, blocks were stained with the antibody manually, using heat induced epitope retrieval method. After washing and blockage of endogenous peroxidase, the slides were incubated with the primary antibody, followed by detection with a streptavidin-biotin immunoenzymatic antigen system. Positive and negative controls were also included. The slides were examined for the histopathological features of the biopsy as well as immunohistochemical characteristics of intraepithelial lymphocytes.

Data was analysed using SPSS version 21. Frequency of clinical symptoms, histological features and immunohistochemical results, was calculated.

**RESULTS**

Fifty patients above the age of 14 years were included in the study. Maximum patients (n=17, 34%) were from the age group 21 - 30 years. There were eleven (22%) patients in 31 - 40 years age group. Eight patients were from age group 14 - 20 years and their percentage is 16%. Equal number (n=7, 14%) of patients were from age groups 41 - 50 years and 51 - 60 years.

Forty-two (84%) patients were males and the remaining 08 (16%) were females. All patients presented with chronic diarrhoea. Among these patients, 12 (24%) patients had iron deficiency anaemia, 9 (18%) had weight loss, 2 (4%) had altered bowel habits, and one (2%) had abdominal distention. Patients with anemia mostly showed partial villous atrophy. Around 4 (8%) biopsies also contained jejunal mucosa while the rest were from duodenal mucosa. The mean number of received fragment was five. Forty-two (84%) cases were received with more than four mucosal fragments. A total of 21 (42%) cases showed positive serology for anti-tissue transglutaminase antibody.

Intraepithelial lymphocytes were counted in 100 enterocytes. Varying number of intraepithelial lymphocytes were seen in all the cases. Highest number of intraepithelial lymphocytes was 72/100 enterocytes and the lowest number was 30/100 enterocytes with a mean number of 39.6. Distribution of all intraepithelial lymphocytes was crescento, i.e. higher number of lymphocytes from the base to the tip of the villous (Figure 1). Out of 50 patients, crypt hyperplasia was seen in 39 (78%) cases while it was absent in 11 (22%) cases. Out of 50 cases, 13 (26%) were showing focal atrophy, while 32 (64%) were showing partial atrophy and 5 (10%) were showing complete atrophy (Table I).

Varying degree of inflammation was seen. Twenty-four (48%) cases showed moderate inflammation while 13

Table I: Number and percentage of cases in each category of modified Marsh criteria and other microscopic parameters.

<table>
<thead>
<tr>
<th>Modified marsh criteria</th>
<th>Raised intraepithelial lymphocytes (n=41)</th>
<th>Crypt hyperplasia (n=39)</th>
<th>Villous atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>82%</td>
<td>78%</td>
<td>3a (Focal)</td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=32)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Percentage of cases</td>
<td>26%</td>
<td>64%</td>
<td>10%</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Other microscopic features</th>
<th>Mucosal edema (n=23)</th>
<th>Goblet cell depletion (n=37)</th>
<th>Epithelial damage</th>
<th>Epithelium shape</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Focal (n=33)</td>
<td>Diffuse (n=4)</td>
<td>Tall columnar (n=24)</td>
<td>Low columnar (n=9)</td>
</tr>
<tr>
<td>Percentage of cases</td>
<td>46%</td>
<td>74%</td>
<td>66%</td>
<td>8%</td>
</tr>
</tbody>
</table>

**Figure 1:** Photomicrograph showing increased intraepithelial lymphocytes in flattened villous (H&E x400).

**Figure 2:** Photomicrograph showing CD3 positive intraepithelial lymphocytes in flattened villous (H&E x400).
(26%) each showed mild and severe inflammation. The inflammatory infiltrate was mostly lymphoplasmacytic in nature. Other histological features and their percentage were also noted such as shape of epithelium, epithelial damage, mucosal edema and goblet cell depletion (Table I).

Immunostain CD3 was positive for intraepithelial lymphocytes in all the 50 cases (Figure 2), while CD20 immunostain was positive in focal areas where there is lymphoid follicle formation with germinal centre (n=19, 38%). Not much difference was seen in count of intraepithelial lymphocytes on immunohistochemistry and routine H&E stain.

**DISCUSSION**

The present study found the peak age of adult celiac disease between 21 - 30 years. The second largest group fell in the age group of 41 - 50 years. According to a recent study conducted in Malaysia, it showed that approximately 1.25% burden of celiac disease falls on young adult in the 24 - 30 years age group. In contrast, burden of this disease lies in the age group above 60 years in Canada. Gender distribution is variable in different parts of the world. While some geographical areas show equal division of male as well as female preponderance, UK shows a clear female predominance. In China, most patients with adult celiac disease were males.

All patients presented with chronic diarrhoea, along with iron deficiency anaemia in 24% of patients. A study in Canada emphasised on the importance of relationship between anaemia and celiac disease, thus establishing it as clinical manifestation of well-developed disease process.

Serology-based studies conducted in Canada showed that celiac disease was diagnosed more frequently partly owing to increased small intestinal biopsies performed in sero-positive patients. According to them, around 84% of cases were received with more than 4 mucosal fragments for histomorphological examination. Data from Brazil recommended maximum of 6 duodenal mucosal fragments from the first part and distal portion of small intestine. However, 4 mucosal fragments are enough to make a diagnosis in 100% of cases.

The highest number of intraepithelial lymphocytes was 72/100 enterocytes and the lowest number was 30/100 enterocytes with a mean value of 39.6 in this study. The maximum number of intraepithelial lymphocytes was 77 with a mean of 20, according to a study in Australia. Crypt hyperplasia is the first architectural change in development of mucosal lesion which is initiated by increased number of intraepithelial lymphocytes.

Similarly, crypt hyperplasia was found in 78% of cases in this study. The systematic description of histopathological features in celiac disease by Marsh is well recognised and is mainly used nowadays. In Spain, adults presenting with celiac disease had predominantly partial villous atrophy along with lymphocytic enteritis, which is consistent with the finding here. A varying degree of inflammation was present. Infiltrate was mostly lymphoplasmacytic type. Similar observations were recorded in Canada. In Slovakia, apart from villous atrophy, various other features were appreciated in mucosal biopsy including goblet cell depletion, pseudostratification of nuclei, and loss of normal shape of enterocytes. In the present study too, the most common type of epithelium was the tall columnar type, followed by pseudostratification. Goblet cell depletion was seen in majority of cases.

Immunohistochemistry was applied for intraepithelial lymphocytes, using CD3 and CD20 immunostains. All of the cases showed positive results with CD3, establishing the fact that intraepithelial lymphocytes were of T-cell origin. The CD20 (B-lymphocytes) showed positivity in areas with lymphoid follicle formation in 19 (38%) of cases. All the intraepithelial lymphocytes were present in crescendo-pattern of distribution. A study in the US showed that the lymphocytes present in epithelium are most CD3/CD8 positive while few are CD3/CD8 negative; and lymphocytes seen in lamina propria are CD20 positive. Although it is a common belief that lymphocytes increase in lateral aspect of villous rather than the tip of villous, it was later found that intraepithelial lymphocytes are concentrated on both lateral aspect as well as tip of villous, which is consistent with the findings in this study. Small intestinal biopsies comprising of minimum 4 mucosal fragments should be recommended in patients with negative serology having high suspicion of celiac disease before incorporating gluten-free diet in treatment plan.

**CONCLUSION**

It is concluded from this study that maximum adult patients, suspected of celiac disease in our setup, belong to age group of 21 - 30 years with males being affected more frequently than females. The common presentation was iron deficiency anaemia along with chronic diarrhoea. Majority of the cases showed partial villous atrophy along with lymphocytic enteritis in almost all cases. Immunohistochemical marker analysis showed that CD3 was positive in intraepithelial lymphocytes, thus establishing its T-cell origin.

**REFERENCES**


