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Original Article

Adults with Celiac Disease: Histopathological and Immunohistochemical Analysis of Small Intestinal Biopsies

Mohammad Manzoor[1*], Moeez Maqbool[2], Adnan Sarwar[3], Mohibullah Khan[3], Inam-u-Ilah[4], Hina Mir[5], Bakhtawar Batool[6], Sudhair Abbas Bangash[7], Fares M Muthanna[8]

[1]Pathology Department, Bacha Khan Medical College Mardan, Pakistan
[2]Cavan General Hospital Cavan, Ireland
[3]Pak International Medical College, Peshawar, Pakistan
[4]Department of Food Science, The University of Haripur, KPK, Pakistan
[5]Department of Biochemistry, Shaheed Benazir Bhutto Women University, Peshawar, Pakistan
[6]Department of Zoology, Quaid I Azam University, Islamabad, Pakistan
[7]Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan
[8]Department of Pharmaceutical Care, School of Pharmacy, Walailak University, Nakhon Si Thammarat, Thailand

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***Corresponding Author:**
Mohammad Manzoor
Pathology Department, Bacha khan Medical College, Mardan, Pakistan
mohammadmanzoor418@gmail.com

ABSTRACT

Celiac disease is the most common cause of malabsorption, which is characterized by a sensitivity to the stored protein gluten, which can be found in wheat, barley, and rice **Objective:** In this study, we evaluated the immunohistochemical and micromorphological techniques for analyzing small intestinal biopsies from adults suspected of celiac disease. **Method:** The study was carried out at the Department of Histopathology, Lady Reading Hospital, Peshawar, from December 2018 to August 2019. Fifty (50) specimens of small intestinal mucosal biopsies were examined in adult celiac disease patients over the age of 14 years. Modified Marsh Criteria were used to record their histomorphology data. Immunohistochemistry was used to determine the kind of intraepithelial lymphocytes. H&E staining and CD3 and CD20 immunostaining were used to count intraepithelial lymphocytes. **Results:** Seventin (34%) of patients were between the ages of 21 and 30 years, and 22% were between the ages of 41 and 50. Out of total 50 cases, males were 42 (84%). Thirteen (26%) of the cases had focal villous atrophy, 32 (64% of the cases) had partial villous atrophy, and 5 (10%) had total villous atrophy. Antibody to tissue transglutaminase was found to be positive in 21 (42%) of the patients. In all 50 cases, the CD3 immunomarker indicated intraepithelial lymphocytes, while the CD20 immunomarker showed localized positivity in lymphoid follicle development. On both H&E stain and immunostaining CD3 and CD20, the number of intraepithelial lymphocytes was determined to be nearly identical (with a difference of 3 - 4 lymphocytes). **Conclusion:** Males between the ages of 21 and 30 were the most affected. Partial villous atrophy and lymphocytic enteritis were the most common histological changes. All intraepithelial lymphocytes were distributed in a crescendo-like manner.

INTRODUCTION

Celiac disease is the most common cause of malabsorption, which is characterized by a sensitivity to the stored protein gluten, which can be found in wheat, barley, and rice[1].It was once thought to be a child's condition, but it is now being detected increasingly frequently in adults and the elderly[2]. It is described as a genetically vulnerable individual's excessive immunological reaction to consuming gluten [3]. It affects about 1% of the world's population, but current research shows that its prevalence is increasing in different geographical locations, owing to improved diagnostic methods and public awareness [4]. Anemia, chronic diarrhea, bloating, flatulence, stomach pain, and changed bowel habits are all common gastrointestinal issues. Adults

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typically present in their third or fourth decade of life, while persons over the age of 34 are more likely to do so [5]. There isn't a lot of information about morphological and immunohistochemical markers studies in this area. the several types of intestinal lesions that might cause celiac disease in adults in Pakistan. This may be due to underdiagnosis and therapy based on guesswork, as serological testing, gastric endoscopy, and histopathology of intestinal sample facilities are still insufficient even in tertiary care centers. The actual prevalence of this group of disorders in Pakistan's adult population is unknown, and little clinical data on the disease's presentation has been reported[6].In this study, we explored how the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition criteria can be used to emphasize the importance of diagnosis in adult patients with chronic diarrhea. The article aimed to see what the histopathological signs of malabsorption syndrome were in an adult Pakistani population with celiac disease.

METHODS

From December 2018 to August 2019, at the Department of Histopathology, Lady Reading Hospital conducted a descriptive study. A total of 50 patients over the age of 14 were enrolled in the study. All small intestine mucosal biopsies from adult celiac disease patients (aged 14 and up) were included. The study did not include mucosal biopsies or autolyzed tissue samples from individuals under the age of 14. Proformas were used to capture clinical data. The biopsies were taken from patients were fixed in 10% formalin for 24 hours. The samples were then subjected to the standard procedure of tissue grossing, processing, paraffin embedding, and sectioning. Hematoxylin and Eosin were used to stain the slides, and two slides from each case were stained for the immunohistochemical markers CD3 and CD20. Blocks were manually stained with the antibody for immunohistochemistry using the heat-induced epitope retrieval procedure. The slides were washed, and endogenous peroxidase was blocked before being treated with the primary antibody and detected using a streptavidin-biotin immunoenzymatically antigen system. There were both positive and negative controls. The biopsy's histopathological traits, as well as the immunohistochemistry properties of intraepithelial lymphocytes, were studied on the slides. Data were analyzed using SPSS version 21. The frequency of clinical symptoms, histological features, and immunohistochemical results were calculated.

RESULTS

Out of total 50 cases, the majority of patients (n=17, 34%) were between the ages of 21 and 30. The age group of 31 to 40 years old had 11 cases (22%). Eight of the patients were between the ages of 14 and 20, accounting for 16% of the total. A similar proportion of patients (n=7, 14%) were in the age categories 41 - 50 years and 51 - 60 years.Forty-two patients (84%) were males, whereas 8 (16%) patients were females. All of the patients had persistent diarrhea. Twelve patients (24%) had iron deficiency anemia, 9 (18%) experienced weight loss, 2 (4%) had abnormal bowel habits, and one (2%) had abdominal distention among these patients. The majority of anemia patients had partial villous atrophy. Around 4% of biopsies (8%) also contained jejunal mucosa, with the remainder being duodenal mucosa. The average number of fragments received was five. More than four mucosal fragments were found in 42 (84%) of the cases. Twenty-one (42%) of the patients had antibodies against tissue transglutaminase. In 100 enterocytes, intraepithelial lymphocytes were detected. All of the cases had a different quantity of intraepithelial lymphocytes. With a mean number of 39.6, the highest number of intraepithelial lymphocytes was 72/100 enterocytes and the lowest number was 30/100 enterocytes. All intraepithelial lymphocytes were distributed in a crescendo pattern, with a higher quantity of lymphocytes from the base to the tip of the villous tube (Figure 1). Crypt hyperplasia was detected in 39 (78%) of the 50 patients, while it was absent in 11 (22%) of the instances. Thirteen (26%) of the 50 patients had focal atrophy, 32 (64%) had partial atrophy, and five (10%) had full atrophy. A varying degree of inflammation was seen. Twenty-four (48%) cases showed moderate inflammation while13 (26%) each showed mild and severe inflammation. The inflammatory infiltrate was mostly lymphoplasmacytic innature. Other histological features and their percentage were also noted such as the shape of epithelium, epithelial damage, mucosaledema, and goblet cell depletion.CD3 immunostaining revealed intraepithelial lymphocytes in all 50 instances (Figure 2), while CD20 immunostaining revealed lymphoid follicle development with the germinal center in localized regions (n=19, 38 percent). On immunohistochemistry and conventional H&E stain, there was little variation in the number of intraepithelial lymphocytes.

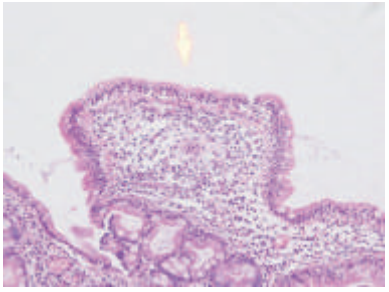

Figure 1: Photo micrograph showing increased intraepithelial

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lymphocytes in flattened villous(H&Ex400)

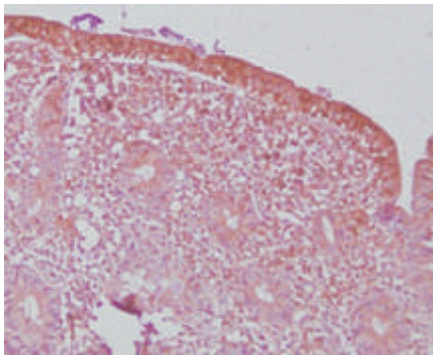

Figure 2: Photo micrograph showing CD3 positive intra epithelial lymphocytes in flattened villous(H&Ex400).

DISCUSSION

The current study observed that adult celiac disease peaks between the ages of 21 and 30. The second-largest group was between the ages of 41 and 50. According to a recent study conducted in Malaysia, young adults aged 24 to 30 years bear around 1.25 percent of the burden of celiac disease. In Canada, however, the condition is most prevalent in people over the age of 60[7]. In many places of the world, gender distribution varies. While certain geographical locations have an equal male and female preponderance, the United Kingdom has a definite feminine predominance [1]. The majority of adult celiac disease patients in China were men [8]. All of the patients had chronic diarrhea, and 24 percent of them had iron deficiency anemia. An important link between anemia and celiac disease was highlighted in a Canadian study, establishing anemia as a clinical manifestation of a well-developed disease process [9]. Celiac disease was detected more frequently in Canada, thanks to an increase in small intestine biopsies performed in seropositive patients, according to serology-based studies. According to them, 84 percent of patients received for histo-morphological examination had more than four mucosal fragments[9]. According to Brazilian research, a maximum of six duodenal mucosal fragments from the initial and distal portions of the small intestine should be used. In 100% of cases, however, four mucosal fragments are sufficient to provide a diagnosis. According to serology-based studies, Celiac disease was found more frequently in Canada as a result of an increase in small intestinal biopsies conducted in seropositive individuals. According to them, more than four mucosal fragments were found in 84 percent of the patients who were sent for histomorphology investigation. 8 According to Brazilian research, no more than six duodenal mucosal fragments from the small intestine's early and distal regions should be employed. Four mucosal fragments, on the other hand, are enough to provide a diagnosis in 100% of cases [10,15]. Marsh's systematic description of celiac disease histological characteristics is well-known and widely used today [11,16]. Adults with celiac disease in Spain exhibited mostly partial villous atrophy and lymphocytic enteritis, which is consistent with the findings presented [12]. Inflammation of varying degrees was found. The lymphoplasmacytic infiltrate was the most common. In Canada, similar observations were made [13]. Apart from villous shrinkage, mucosal biopsy in Slovakia revealed goblet cell depletion, pseudo stratification of nuclei, and loss of normal shape of enterocytes, among other things [1]. The tall columnar epithelium was the most common kind of epithelium in this study as well, followed by pseudo stratification. In the vast majority of cases, goblet cell depletion was seen.CD3 and CD20 immuno-stains were used for intraepithelial lymphocytes in immunohistochemistry. All of the cases tested positive for CD3, indicating that the intraepithelial lymphocytes were T-cell-derived. In 19 (38 percent) of the patients, CD20 (B-lymphocytes) were positive in lymphoid follicle development sites. All of the intraepithelial lymphocytes were distributed in a crescendo pattern. A study from America concluded lymphocytes in the epithelium are mostly CD3/CD8 positive, with only a few CD3/CD8 negative cells; lymphocytes in the lamina propria are CD20 positive [14]. Although it is commonly assumed that lymphocytes increase in the lateral aspect of the villous rather than the tip, it was later discovered that intraepithelial lymphocytes are focused on both the lateral and tip of the villous,20 which is consistent with the results of our investigation. Before introducing a gluten-free diet into a treatment plan, patients with negative serology and a strong suspicion of celiac disease should have small intestine biopsies with at least four mucosal fragments taken.

CONCLUSION

According to the findings of this study, the majority of adult patients suspected of celiac disease in our setting are between the ages of 21 and 30, with males being affected more commonly than females. Iron deficiency anemia and persistent diarrhea were the most common symptoms. The majority of the cases had partial villous atrophy, which was virtually always accompanied by lymphocytic enteritis. CD3 was positive in intraepithelial cells, indicating a T-cell origin, according to immune histochemical marker analyses.

REFERENCES

[1] Iftikhar R, Jamal S, Zafar A, Saadia et al, & surgeons-Pakistan. Histopathological and immunohistochemical analysis of small intestinal biopsies in adults suspected of celiac disease. 2016,26(10), 827-830.

DOI:https://doi.org/10.54393/pbmj.v5i1.319

[2] Vilppula A, Kaukinen K, Luostarinen et al,.Increasing prevalence and high incidence of celiac disease in elderly people: a population-based study.2009, 9(1), 1-5. doi.org/10.1186/1471-230X-9-49
[3] Hopper AD, Hadjivassiliou M, Butt et al, Adult coeliac disease. 2007,335(7619), 558-562. doi.org/10.1136/bmj.39316.442338.AD
[4] Rubio-Tapia A, Ludvigsson JF, Brantner TL, et al,The prevalence of celiac disease in the United States.2012, 107(10), 1538-1544.doi.org/10.1038/ajg.2012.219
[5] Yap TW-C, Chan W-K, Leow AH-R, Azmi AN, et al., Prevalence of serum celiac antibodies in a multiracial Asian population-a first study in the young Asian adult population of Malaysia.2015, 10(3), e0121908.doi.org/10.1371/journal.pone.0121908
[6] Abbas Z, Raza S, Yakoob J, Abid, et al, Pakistan, S. Varied presentation of celiac disease in Pakistani adults.2013, 23(7), 522.
[7] Lerner A, & Matthias TJ, et al,. Increased knowledge and awareness of celiac disease will benefit the elderly. 2015,3, 112-114. doi.org/10.12691/ijcd-3-3-6
[8] Jiang L-l, Zhang B-l, & Liu Y-s, et al, Is adult celiac disease uncommon in Chinese? ,2009, 10(3), 168-171. doi.org/10.1631/jzus.B0820164
[9] Freeman HJJI, et al,. Small intestinal mucosal biopsies for case-finding in celiac disease.2015, 3, 50-52. doi.org/10.12691/ijcd-3-2-3
[10] KOTZE LMdS, Mallmann A, et al, Reproductive aspects in Brazilian celiac women.2020, 57, 107-109 doi.org/10.1590/s0004-2803.202000000-18
[11] Villanacci V, Ceppa P, Tavani E, et al, Coeliac disease: the histology report. 2011,43, S385-S395. doi.org/10.1016/S1590-8658(11)60594-X
[12] Vivas S, Vaquero L, Rodríguez-Martín L, et al, & therapeutics. Age-related differences in celiac disease: Specific characteristics of adult presentation.2015, 6(4), 207. doi.org/10.4292/wjgpt.v6.i4.207
[13] Freeman HJJG, & liver, Adult celiac disease and its malignant complications.2009, 3(4), 237.doi.org/10.5009/gnl.2009.3.4.237
[14] Hudacko R, Kathy Zhou X, & Yantiss,et al,.Immunohistochemical stains for CD3 and CD8 do not improve the detection of gluten-sensitive enteropathy in duodenal biopsies.2013, 26(9), 1241-1245. doi.org/10.1038/modpathol.2013.57
[15] Freeman HJ, Role of biopsy in diagnosis and treatment of adult celiac disease. Gastroenterol Hepatol Bed Bench. 2018;11(3):191-196.
[16] Narang V, Jindal A, Singh A, Varun Mehta BG, Sood N, Sood A, Diagnostic utility of multiple site duodenal biopsies in celiac disease. Indian J Pathol Microbiol. 2021 Jun;64(Supplement):S73-S77. doi: 10.4103/IJPM.IJPM_797_20.