SPECTRUM OF HISTOPATHOLOGICAL PATTERNS OF DUODENAL BIOPSIES IN PATIENTS WITH UNEXPLAINED ANAEMIA AND CHRONIC DIARRHEA AND THEIR CORRELATION WITH ENDOSCOPIC FINDINGS AND SEROLOGICAL MARKERS

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ABSTRACT

Objective: To evaluate different histopathological patterns and correlate them with indications, findings of Esophagogastroduodenoscopy Esophago-gastro-duodenal (EGD) and serological markers in patients presenting of unexplained anemia and chronic diarrhea.

Study Design: Cross sectional study.

Place and Duration of Study: Departments of Pathology and Gastroenterology, Combined Military Hospital Lahore Pakistan, from Jul to Dec 2020.

Methodology: Histopathological patterns of endoscopic duodenal biopsies, submitted for evaluation of unexplained anemia and chronic diarrhea were studied. Hemoglobin and anti-tTG levels were recorded. Adults with history of unexplained diarrhea and anaemia were included. Biopsies with malignant diagnosis or unfit for evaluation were excluded. Histopathological patterns were correlated with indications and findings of Esophagogastroduodenoscopy and serological markers of celiac disease.

Results: The most common indication for Esophagogastroduodenoscopy in 145 patients was chronic diarrhea. Upper gastrointestinal endoscopy in 2/3rd of patients revealed no pathology. Histopathological patterns of duodenal biopsies revealed only 15% cases suggestive of celiac disease. Only 12 patients were suggestive of celiac disease both on Esophagogastroduodenoscopy and histopathology combined. Half of patients with anti tTG level >100 u/ml, showed histopathological features of celiac disease on. There was no correlation between histopathological patterns, indications of Esophagogastroduodenoscopy, morphology of Esophagogastroduodenoscopy and serological markers of celiac disease.

Conclusion: Indications for Esophagogastroduodenoscopy, Esophagogastroduodenoscopic findings and histopathological patterns cannot diagnose celiac disease alone.

Keywords: Anaemia, Celiac disease, Chronic diarrhoea, Esophagogastroduodenoscopy.

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INTRODUCTION

Esophagogastroduodenoscopy endoscopy (EGD) is a caveat in diagnosing many gastrointestinal diseases. It allows visual inspection and an opportunity to obtain specimens for histopathological evaluation. Evaluation for anaemia and chronic diarrhoea are two common indications where EGD with duodenal and/or gastric biopsies is advised. Gastrointestinal causes of anemia include are variety of diseases along with the alimentary tract and liver diseases. Duodenal biopsies are recommended where there is suspicion of malabsorption as a contributory cause of anaemia.[1] Clinical findings, inspection of duodenal mucosa and its correlation with serological and histopathological findings of endoscopic biopsies are all utilized to reach at definite aetiology of malabsorption and anaemia.[2,3] Anaemia has been defined as low haemoglobin with different cut off values in literature.[4] Approach to evaluation of anaemia includes estimation of hematological parameters most important being mean corpuscular volume (MCV). Among the three types of anaemia based on MCV, duodenal biopsies are most helpful in evaluation of microcytic anaemia with low iron levels indicated by low serum ferritin.[5] Apart from microcytic anemia, duodenal biopsies are an important tool for evaluation of chronic diarrhoea. Chronic diarrhoea is classically defined as diarrhoea lasting >4 weeks.[6,7] It results in malabsorption syndrome and anaemia which can be either microcytic or macrocytic.

Celiac disease is an important cause of malabsorption and anemia. Iron deficiency anaemia is a significant feature of celiac disease. Patients can also present with magaloblastic anemia as a late manifestation of disease due to malabsorption of vit B12 and folate irrespective of age, sex and ethnicity.[8] Endoscopic fin-

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dings provide clue for the disease, where as histopathologic features of the celiac disease manifest infiltration of intra epithelial lymphocytes, hypertrophy of crypt leading to villi atrophy.[9] Objective of our study was to correlate histopathological findings of biopsies with indications of EGD, findings of EGD and serological markers for diagnosing celiac disease.

METHODOLOGY

This cross-sectional study was carried out at the departments of Pathology and Gastroenterology, Combined Military Hospital (CMH) Lahore Pakistan, from July to December 2020. Approval was taken from ERB (ethics review board) (No:217/2020). All endoscopic duodenal biopsies submitted to Histopathology department of CMH Lahore for evaluation of anemia and chronic diarrhea were retrieved from lab records. The study sample size was estimated using a single population proportion formula using Australian Bureau of Statistics online sample size calculator, which was calculated with following assumptions: 95% confidence level, 5% margin of error and 0.7% expected proportion of celiac disease on biopsy among patients presenting with chronic diarrhea, based on a similar study findings by Singh *et al*[10]. Given these assumptions the required sample size was determined to be 27. As the sampling technique used was consecutive, sample size was increased to 145 patients. Biopsies fulfilling inclusion criteria were selected. Endoscopic findings were confirmed from endoscopy record book. Hemoglobin levels and anti-tTG levels of these patients were recorded. Consent for using lab data for research purpose was obtained from patients while submitting histopathology specimens.

Inclusion Criteria: The adult patients, aged >12 years having history of chronic diarrhea, anaemia and chronic diarrhea along with anaemia for duration of >6 months were included in the study

Exclusion Criteria: Patients who did not consent for usage of their specimens for research work were not included in the study. Biopsies showing diagnosis of malignancy, biopsy material too scanty (<5 villi available) or blocks not available and/or showing processing/fixation artifacts were excluded.

All patients had underdone oesophago-gastroduodenoscopy (EGD) through magnification Olympus gastroscope (GF 180, Olympus, Japan) by gastroenterologist. Four specimen biopsies, 2 from 1st and 2 from 2nd part of duodenum were taken. Biopsies were fixed in 10% buffered formal saline for 4-8 hours and then processed in Leica automated processor for 6 hours. Specimens were embedded in paraffin and blocks were made. Five micron thick sections were cut by microtome and stained by hematoxylin and eosin. Sections were mounted on slides and cover slips were applied for microscopic examination. Histopathological patterns of biopsy specimens were studied under microscope by two different histopathologists independently. Difference of opinion was discussed to reach at final opinion in a few case.

Results were entered and anlysed using SPSS-25. Frequencies and percentages were obtained for indications and findings of EGD and histopathology findings. Correlation of indications, EGD findings and serological findings with histopathological patterns were calculated by Spearman method. The *p*-value of ≤5 was considered significant.

RESULTS

A total of 168 duodenal biopsies were received, but our study included only 145 patients who fulfilled inclusion criteria. Among these, 101 (69.7%) were male and 44 (30.3%) were female patients. Age range was 13-72 years with mean age 39.8 ± 15.8 years. The most common indication for EGD in our study was chronic diarrhoea (61%). Frequencies of indications for EGD and histopathological findings are shown in the Table-I.

Table-I: Findings of Esophagogastroduodenoscopy (EGD) and Histopathological pattern of biopsies (n=145).

Esophagogastroduodenoscopy (EGD) Endoscopic Findings	n (%)
Typical Findings of Celiac Disease	31 (21.4)
Non-specific Findings	19 (13.1)
Unremarkable study	95 (65.5)
Histopathologic Findings	
Diffuse severe villous abnormality with crypt hyperplasia	23 (15.9)
Variable villous abnormality with crypt hypoplasia	2 (1.4)
Non-specific variable villous abnormality	26 (17.9)
Variable villous abnormality with specific diagnosis	16 (11)
Unremarkable study	78 (53.8)

On upper GI endoscopy 2/3rd (95/145) of patients did not reveal any pathology. Histopathology of duodenal biopsies suggestive of celiac disease was found in only 23/145 (15%) of cases. Microscopy was unremarkable in 78/145 (53.7%) of cases. Indications for EGD were anaemia in 57(39.3%), chronic diarrhea in 62 (42.8%) and combined chronic diarrhea and anaemia in 26 (17.9%) cases of study population. There was a negative correlation of histopathological findings

with indications of Esophagogastroduodenoscopy and Esophagogastroduodenoscopic findings by Pearson's coefficient r=0-.003 and -0.15 respectively, shown in the Table-II.

Table-II: Pearson's correlation of histopathological findings.

Parameters	Pearson Correlation Co-efficient & *p*-value	Values
Age (In years)	Pearson Correlation Co-efficient	0.080
	p-value	0.660
Gender	Pearson Correlation Co-efficient	0.034
	p-value	0.926
Indication	Pearson Correlation Co-efficient	-0.003
	p-value	0.160
Esophagogastro duodenoscopic findings	Pearson Correlation Co-efficient	-0.105
	p-value	0.000
Heamoglobin	Pearson Correlation Co-efficient	0.211
	p-value	0.022
Anti tTG Level	Pearson Correlation Co-efficient	-0.339
	p-value	0.130

DISCUSSION

Celiac disease is autoimmune in nature. It is a gluten-sensitive enteropathy in genetically predisposed individuals to gluten intolerance. Gluten, a toxic component of cereal grains, affects proximal intestine more than distal[11]. The small bowel mucosal responses to injury are limited. Recognition of these response patterns can be useful in differential diagnosis. In term'' severe villous abnormality'' intestinal mucosa is flat showing no villi. In term ''variable villous abnormality'' the villi are either only focally flat or are less than flat. Pattern of abnormal small-bowel architecture are: 1) diffuse severe villous abnormality and crypt hyperplasia[2]. Variable villous abnormalities and crypt hypoplasia[3]. Non-specific variable villous abnormality, usually not flat[4]. Variable villous abnormalities with specific diagnostic changes[12]. Diagnosis of the disease involves high index of suspicion and correlation of EGD findings and specific serologic markers with histopathological findings of duodenal mucosa. Diagnosis of celiac disease can be made if high titres of celiac specific serologic markers are found in immune-competent patients with diffuse villous abnormality and crypt hyperplasia on microscopy of duodenal biopsy.

We studied 145 patients of unexplained anemia and chronic diarrhea, who had undergone duodenal biopsies. They were 69.7% males and 30.3% females. Male to female ratio was 2.3:1. Reported data on celiac disease shows male to female ratio to be 1:2 with some of the studies having consensus of 1:1[13,14].

Analysis of indications of EGD with histopathological patterns revealed that on microscopy, findings suggestive of celiac disease are mostly found in anaemic patients (11%) as compared to patients with chronic diarrhea (8%) and indications of EGD and histopathology have negative correlation with Pearson's coefficient r=-0.003. Analysis of EGD findings with histopathology revealed that only (8.27%) patients were suggestive of celiac disease on both macroscopy and microscopy (Figure-2). Histopathology and esophagogastroduodenoscopic findings have strong negative correlation with Pearson's coefficient r=-0.105. Anti-tTG serology was available for only 48/145 (33.1%) patients and 31/48 patients had anti-tTG level <20u/ml, out of which 4 patients had findings of celiac disease on histopathology, 9/48 had anti tTG level between 20-100 u/ml, out of which 2 patients were suggestive of celiac disease on histopathology. Eight out of forty eight patients had anti-tTG level >100u/ml out of which 4 patients were confirmed to have celiac disease on histopathology. Age range of patients showing classical features of celiac disease on histopathology was 19-70 years in our study. However most of the patients were in the age range of 25-50 years. Male to female ratio was 2.3:1 in our study.

The frequency of celiac disease reported in European counties is 1% with the highest prevalence in Finland (2.4%). In Asia, a particularly high prevalence of celiac disease has been reported from Indian Punjab where wheat has been used as a staple diet for generations[15]. In present study common indications of upper GI endoscopy were chronic diarrhoea (42.8%), and anaemia (39.3%), and chronic diarrhoea along with anaemia, of which 13%, 19.29% and 15.38% patient were diagnosed with celiac disease on histopathological examination, respectively. This reveals that celiac disease is mostly found in anaemic patients (19.29%) in our study, while worldwide prevalence of celiac disease in patients with iron deficiency anaemia varies from 0-5%. Grisolano and colleagues reported that its frequency was 8.7% (n=103)[16,17]. Broide, *et al* have recommended that patients with unexplained iron deficiency anaemia should be worked up for celiac disease by histopathology of biopsies[18]. In our study, 95 (65.5%) patients had unremarkable findings on EGD, but 10 (6.8%) patients were diagnosed as celiac disease on histopathology. It reveals that EGD alone has lower sensitivity and absence of endoscopic findings does

not exclude celiac disease. It is therefore recommended that microscopic examination of biopsies should be done for conclusive diagnosis. Similar findings have been reported by Manpreet *et al* and other international studies as well[19-21]. Only 12.5% patient with anti tTG level >100 u/ml, had unremarkable study on microscopy while 50% patients had celiac disease on histopathology. On the other hand, 48.3% patients with anti tTG level <20u/ml had unremarkable study on microscopy and 13% were diagnosed with celiac disease on histopathology.

According to international studies, IgA levels are low in 0.2% of general population and 2% of celiac disease patients who have diagnosis of severe villous abnormality with crypt hyperplasia on histopathology but negative serology should be worked up for other diseases which also cause mucosal abnormalities or should be checked for IgA, IgG- tTG or IgGD GP levels before conclusion[22,23].

Our findings showed no positive correlation between clinical symptoms, indications and findings of EGD, celiac serology and histopathological study. Our results are in agreement with a study conducted in Iran[24]. Therefore, it is recommended that all patients with unexplained anaemia, chronic diarrhoea and weight loss should undergo EGD. Biopsies for histopathological examination must be taken to reach at a conclusive diagnosis. However, our study has certain.

CONCLUSION

Indications for EGD, EGD findings and histopathological patterns can not diagnose celiac disease alone. Higher levels of anti tTG levels are highly suggestive of celiac disease. Patients with unexplained anemia should be worked up with EGD and histopathological evaluation to rule out celiac disease.

LIMITATION OF STUDY

Like a smaller sample size because of COVID-19 pandemic during the whole study period. Male patients were more than females as already explained. In addition, we did not investigate any of the patients for deficiencies of IgA, IgG HLA DQ2/DQ8 levels.

Conflict of Interest: None.

Authors' Contribution

MAY: Concept and design, MAF: Proof reading, NU: Proof reading, BP: Concept, design and data interpretation, AA: Data collection and statistics, JA: Data collection.

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