Osmangazi Tıp Dergisi
Osmangazi Journal of Medicine 2023;

Research Article / Araştırma Makalesi

Investigation of the Frequency and Significance of Gastric Metaplasia in Duodenal Biopsy Materials of Untreated Pediatric Celiac Patients

Tedavi Edilmemiş Pediatrik Çölyak Hastalarının Duodenal Biyopsi Materyallerinde Gastrik Metaplazi Sıklığının ve Öneminin Araştırılması

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Abstract

Gastric (foveolar) metaplasia (GM) in the duodenum occurs in many cases where duodenitis develops histologically. However, few studies have investigated the presence of GM in celiac patients. This study aimed to determine the prevalence of GM and its relationship with clinicopathological parameters in untreated pediatric celiac patients. Duodenal biopsy specimens of 153 pediatric cases were analyzed. MUC5AC immunohistochemical staining was applied to all cases to detect GM. The patient group was evaluated in terms of the disease activity score (DAS), neutrophilic activity score (NAS), eosinophilic infiltration, and presence of intramucosal Brunner glands. The rate of GM was 53.4% in the control group and 98.8% in the patient group. GM was observed as diffuse morphology at a rate of 4.1% (n:3) in the control group and 55% (n:44) in the patient group ($p<0.001$). Diffuse type of GM was more frequent in girls in the patient group ($p=0.03$). When GM (focal/diffuse) age, DAS, NAS, and all histopathological parameters were compared, a statistically significant difference was found between diffuse GM and DAS, and NAS. ($p=0.023$ and $p=0.039$, respectively). The present study considered that duodenal neutrophilia and increased disease activity might play a role in the development of GM in celiac patients. We think that it would be appropriate to include a diffuse type of GM, which can be easily detected by immunohistochemical or histochemical methods, in pathology reports as a finding of histologically active celiac disease.

Keywords: Gastric metaplasia, celiac disease, duodenum, pathology

Özet

Duodenumda gastrik (foveolar) metaplazi (GM), histolojik olarak duodenitin geliştiği birçok durumda ortaya çıkar. Bununla birlikte, çölyak hastalarında GM'nin varlığını araştıran az sayıda çalışma vardır. Bu çalışma, tedavi edilmemiş pediatrik çölyak hastalarında GM prevelansını ve klinikopatolojik parametrelerle ilişkisini belirlemeyi amaçlamaktadır. 153 pediatrik olgunun duodenal biyopsi örneği incelendi. GM'yi saptamak için tüm olgulara MUC5AC immünohistokimyasal boyası uygulandı. Hasta grubu hastalık aktivite skoru (DAS), nötrofilik aktivite skoru (NAS), eozinofilik infiltrasyon ve intramukozal Brunner bezlerinin varlığı açısından değerlendirildi. GM, kontrol grubunda %53.4, hasta grubunda ise %98.8 idi. GM, kontrol grubunda %4,1 (n:3) ve hasta grubunda %55 (n:44) oranında yaygın morfolojide gözlendi ($p<0,001$). Hasta grubundaki kızlarda yaygın GM daha sıktı ($p=0.03$). GM (fokal/yaygın) yaş, DAS, NAS ve tüm histopatolojik parametreler karşılaştırıldığında, yaygın GM ile DAS ve NAS arasında istatistiksel olarak anlamlı fark bulundu. (sırasıyla $p=0.023$ ve $p=0.039$). Bu çalışmada, çölyak hastalarında GM gelişiminde duodenal nötrofili ve artmış hastalık aktivitesinin rol oynayabileceği düşünülmüştür. İmmünhistokimyasal veya histokimyasal yöntemlerle kolaylıkla saptanabilen diffüz tip GM'nin patoloji raporlarında histolojik olarak aktif çölyak hastalığı bulgusu olarak yer almasının uygun olacağı kanaatindeyiz.

Anahtar Kelimeler: Gastrik metaplazi, çölyak hastalığı, duodenum, patoloji

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Received 17.08.2022 Accepted 01.12.2022 Online published 07.02.2023

Canaz F, Oflas D, Yilmaz E, Arik D, Aydemir Y, Baris, Z, Ozen H, Investigation of the Frequency and Significance of Gastric Metaplasia in Duodenal Biopsy Materials of Untreated Pediatric Celiac Patients, Osmangazi Journal of Medicine, 2023;45(2):180-187
Doi: 10.20515/otd.1161843

1. Introduction

Gluten-sensitive enteropathy, known as celiac disease (CD), is an immune-mediated disease that causes malabsorption, characterized by triggering inflammation in the small intestine after exposure to gluten in genetically predisposed individuals (1,2). The prevalence of CD is approximately 1% in the United States. Compared to adults, the worldwide prevalence of biopsy-confirmed CD is higher in children (0.9% vs. 0.5%) (3). The incidence and prevalence of CD in children have been reported to increase gradually in recent years (4). Typical histopathological findings in celiac patients are villus atrophy, crypt hyperplasia, intraepithelial lymphocytosis, and increased lymphoplasmacytic cells in the lamina propria (3,5,6). Gastric (foveolar) metaplasia (GM) in the duodenal mucosa of celiac patients has rarely been reported. GM is characterized by the replacement of non-gastric epithelial cells with cells of the gastric phenotype. The first change in the intestine usually occurs in a superficial region of the villi. Metaplastic cell resembles the mucus-secreting foveolar or gastric pit cells found in superficial sections of glands in the gastric body and antrum (7). Increased gastric acid secretion has been indicated to play a role in the development of duodenal GM (8,9). A study evaluating conditions that may be associated with GM in the duodenum revealed that GM was more common in men with high gastric acid output. GM was not more common in *Helicobacter pylori(H.pylori)*-positive patients, but if the presence was greater in extent, suggesting that the mucosal injury related to active duodenitis in these patients may act as a further stimulus to metaplasia (10,11). This study aimed to determine the incidence of GM and investigate the relationship of GM with clinicopathological parameters in duodenal biopsy materials of pediatric CD.

2. Materials and Methods

Study population

Duodenal endoscopic biopsy material of 153 pediatric patients who applied to the pediatric gastroenterology and hepatology department between 2009 and 2019 was included in the study. 73 duodenal biopsies with endoscopic and histopathological normal appearance were determined as the control group. The celiac patient group consisted of 80 cases whose diagnosis of CD was supported clinically and histopathologically. All biopsy samples in this group were obtained before gluten-free diet therapy. Biopsy materials taken from the bulb and biopsy specimens from patients with a history of concurrent inflammatory bowel disease or a diagnosis of *H.pylori* gastritis were excluded.

Histological analysis

Routine 5 µm thick hematoxylin and eosin-stained sections used for diagnosis were reviewed. The evaluated parameters and the scoring systems used for each parameter are summarized in Table 1. The disease activity score (DAS) was determined as a total score including villous architecture, crypt hyperplasia, lymphoplasmacytic infiltration of the lamina propria, and intraepithelial lymphocyte (IEL) score. The number obtained by dividing the number of IEL per 100 enterocytes by 20 was determined as the IEL score. The neutrophilic activity score (NAS) was determined according to the presence of neutrophilic infiltration, superficial neutrophilic exocytosis, neutrophilic cryptitis, and neutrophilic microabscess in the lamina propria and calculated by summing scores for all parameters (range, 0 to 8). Other parameters were scored according to their presence and extent of eosinophilic infiltration, GM, and intramucosal Brunner glands (2). MUC5AC (clone CLH2, DAKO) immunohistochemical staining was applied to all paraffin blocks to evaluate GM.

Statistical Analysis

Data analysis was performed with IBM SPSS 21 program. Summary values of quantitative (numeric) variables were presented as mean±standard deviation or median (Q1-Q3) values, while qualitative (categorical) variables were as frequency and percentages. The normality of quantitative variables was evaluated with the Shapiro-Wilk test. Mann-Whitney U test was used for two-group comparison when the normality failed. The relationship between qualitative variables was

examined with chi-square tests. Factors affecting CD were investigated with a multiple logistic regression model. P-values less than 0.05 were considered statistically significant.

3. Results

The study population consisted of 2 groups; a patient group consisting of 80 cases with a median age of 8 years (range, 4-11) (57.5% female) and a control group consisting of 73 cases with a median age of 14 years (range, 11-16) (53.4% female). In addition, the rate of cases aged <10 years was 41.2% (n:63), and 58.8% (n:90) were ≥10 years in the entire study group.

Prevalence of GM

The rate of GM was 53.4% (n:66) in the control group and 98.8% (n:79) in the patient group. GM was observed as diffuse morphology at a rate of 4.1% (n:3) in the control group and 55% (n:44) in the patient group (P <0.001) (see Figure 1-2). GM was not observed in one case in the patient group.

Table 1. Histopathologic Parameters and Scoring System

Parameter	Scoring system
DAS	
Villous architecture	0: Normal 1: Mild blunting 2: Moderate blunting 3: Flat
Crypt architecture	0: Normal 1: Hyperplasia 2: Atrophy
Lamina propria lymphoplasmacytic infiltration	0: No increase 1: Mild increase 2: marked increase
IEL score	IEL/100 enterocytes (divided by 20);
NAS	
Lamina propria neutrophilic infiltration	0: None 1: Few foci in the lamina propria 2: Multifocal
Surface neutrophilic exocytosis	0: Absent 1: Few foci 2: Multifocal
Neutrophilic cryptitis	0: Absent 2: Present
Neutrophilic microabscess	0: Absent 2: Present
Eosinophilic infiltration	
Lamina propria eosinophils	Number per HPF: 5 fields counted
Eosinophilic exocytosis	0: Absent or focal 1: Multifocal exocytosis without associated epithelial damage 2: Marked exocytosis with associated epithelial damage and/or eosinophilic microabscesses
Gastric metaplasia	0: Absent 1: Focal (<5% of surface epithelium) 2: Diffuse
Intramucosal Brunner glands	0: Absent 1: Focal 2: Prominent (> 50% of the lamina propria)

Clinicopathological findings and relationship with gastric foveolar metaplasia in the patient group

The median DAS, NAS, IEL, and eosinophil counts in celiac patients were 7.5 (range, 7-8.13), 2 (range, 0-4), 49 (range, 42-54), and 30 (range, 20-49), respectively. Types of GM

in celiac patients were compared according to gender and histopathological findings. Diffuse-type GM proportion was significantly higher in female patients (P =0.03). Diffuse-type GM was observed more frequently in cases with severe villous atrophy, multifocal neutrophilic infiltration, surface neutrophilic exocytosis, neutrophilic cryptitis, and intramucosal Brunner glands (Figure 3). However, there was no statistically significant difference (see Table 2). DAS and NAS scores were significantly higher in the diffuse GM group (P =0.023 and P =0.039, respectively). Nonetheless, no differences were observed according to age, eosinophil count (P =0.419), and IEL count (P =0.366) between types of GM (see Table 3). In multivariate regression, the diffuse-type GM correlated positively with age (≥10 years), and NAS. (see Table 4).

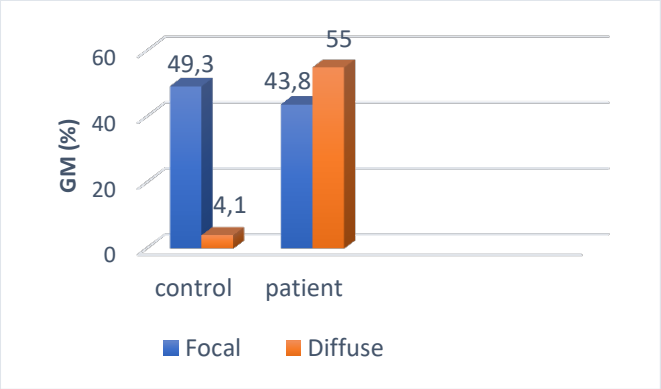

Figure 1. Distribution of gastric metaplasia in groups.

Table 2. Gastric metaplasia and clinicopathological parameters in celiac patients.

GM	Focal (n,%)	Diffuse (n,%)	P value
Gender			**0.03**
Female	13 (37.1%)	32 (72.7%)	
Male	22 (62.9%)	12 (27.3%)	
Villous architecture			0.63
Mild blunting	11 (31.4%)	8 (18.2%)	
Moderate blunting	19 (54.3%)	20 (45.5%)	
Flat	5 (14.3%)	16 (36.4%)	
Crypt architecture			N
Hyperplasia	35 (100%)	44 (100%)	
Lymphoplasmacytic infiltration			0.129
Mild increase	6 (17.1%)	2 (4.5%)	
Marked increase	29 (82.9%)	42 (95.5%)	
Neutrophilic infiltration			0.74
None	15 (42.9%)	14 (31.8%)	
Few foci	7 (20%)	3 (6.8%)	
Multifocal	13 (37.1%)	27 (61.4%)	
Surface neutrophilic exocytosis			0.113
Absent	23 (65.7%)	21 (47.7%)	
Few foci	7 (20%)	8 (18.2%)	
Multifocal	5 (14.3%)	15 (34.1%)	
Neutrophilic cryptitis			0.113
Absent	32 (91.4%)	33 (75%)	
Present	3 (8.6%)	11 (25%)	
Neutrophilic microabscess			N
Absent	35 (100%)	44 (100%)	
Present	0 (0%)	0 (0%)	
Intramucosal Brunner glands			0.766
Absent	14 (40%)	4 (31.8%)	
Focal	19 (54.3%)	27 (61.4%)	
Prominent	2 (5.7%)	3 (6.8%)	

**N: No statistics are computed*

Table 3. Comparison of quantitative variables and gastric metaplasia in celiac patients.

	Focal GM Mean ± SD	Median (min-max)	Diffuse GM Mean± SD	Median (min-max)	P value
Age	7 ± 4	7 (4-10)	8 ± 4	9 (6-11)	0.311
IEL	49 ± 9	45 (42-50)	49 ± 7	50 (45-55)	0.366
DAS	7.06 ± 1.06	7.10 (6.25-8)	7.58 ± 0.93	7.5 (7.18-8.25)	**0.023**
NAS	2 ± 2	2 (0-3)	3 ± 2	3 (0-4)	**0.039**
EC	36 ± 19	30 (20-50)	32 ± 19	30 (20-42)	0.419

**EC: eosinophil count*

Table 4. Parameters correlated with GM in celiac patients

	Logistic Regression Analysis		
Clinicopathological variables	**HR**	**%95 CI**	**P-value**
Age			
<10 age	1		
≥10 age	13.174	3.758-46.182	**<0.001**
Gastric metaplasia			
absent	*	*	**<0.001**
focal	14.583	1.631-134.428	**0.017**
diffuse	198.713	15.627-2526.856	**<0.001**
Neutrophilic activity score	6.310	1.888-21.89	**0.03**

**: The data obtained are not reliable because convergence could not be achieved.*

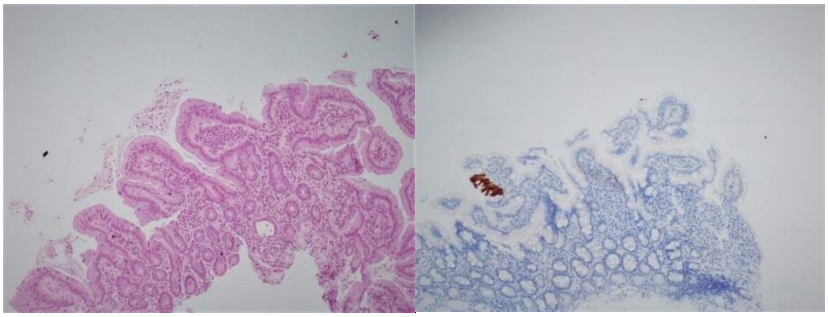

Figure 2. A, Duodenum with normal morphology in the control group, H&E,100x. B, Focal gastric metaplasia was observed with MUC5AC immunohistochemical staining, 100x.

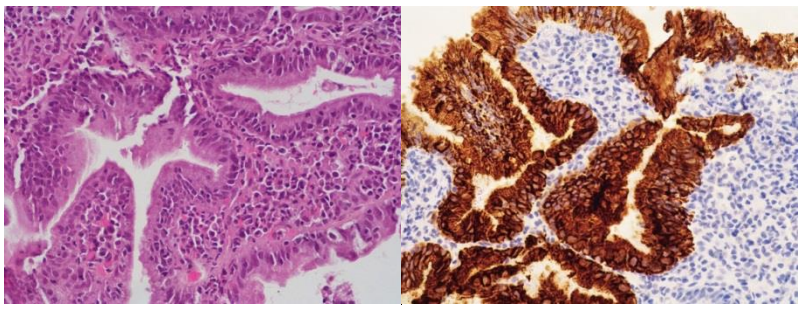

Figure 3. Representative images from celiac disease. A, Duodenal biopsy of a 7-year-old male patient with villous atrophy and intraepithelial lymphocytosis with NAS: 3 (H&E,100x). B, In this case, there is neutrophilic infiltration in the lamina propria and neutrophil exocytosis to the surface epithelium, (H&E, 400x). C, Diffuse gastric metaplasia was observed with MUC5AC immunohistochemical staining, (400x).

4. Discussion

Taylor reviewed gastric heterotopia in the digestive tract in 1927 and described gastric epithelium in 2 duodenal biopsies. In this study performed on necropsies, the condition was observed as slightly raised patches from the surface. Histopathological examination revealed that this area was lined with well-developed gastric epithelium and gastric glands containing both chief and parietal cells (12). In animal studies, GM was associated with duodenal damage and ascites (13). When Harris et al. when compared GM with the control group, they detected more GM in those who developed duodenal ulcers. A positive correlation was determined between gastric output and GM prevalence. GM was reported at a rate of 56% in the control group and 89% in patients with duodenal ulcers. In this study, there was no correlation between GM and *H.pylori* (8). Kreuning et al. determined superficial GM in duodenal bulb biopsies at a rate of 64% in healthy individuals (14). However, lower rates of GM were observed in the control group in other studies (15,16). In another study, GM was detected at a rate of 35% in the group with active duodenitis and 4% in the normal group (17). The literature has reported that this difference may be related to the number of biopsy samples, and the rate would be lower when the biopsy is taken only from the anterior wall, and quadrant biopsy sampling is recommended (8,10). Few studies have evaluated GM in celiac patients and the pediatric age group. Ronald et al. determined GM in pediatric untreated celiac patients at the rate of 70% (n:7) in the proximal part of the duodenum, 67% (n:8) in the distal part, and 68% (n:15) in combination. In this study, this rate in normal healthy biopsies was 0% (n:0), 16% (n:2), and 9% (n:2), respectively (7). In a study performed with celiac patients in the pediatric and adult population, the rate of GM was determined as 21.3% in the entire study group and 17.9% in the total pediatric population (2). In our study, the GM rate was 53.4% in the control group and 98.8% in the celiac patient group. We evaluated GM in two categories as focal and diffuse. GM was observed as diffuse morphology at a rate of 4.1% in the control group and 55% in the patient group ($p<0.001$).

GM is less common in children. Therefore, its etiology is considered to be acquired rather than congenital. (10). The frequency of GM in children increases with age (17). When examining GM in children with CD, Ronald et al. determined the incidence was 68% (n:7) under 3 years of age and 85% (n:6) over 3 years of age. This study observed no significant relationship between age and GM (7). Similarly, in our study, there was no relationship between GM and age in celiac patients. However, in multivariate regression, GM was positively correlated with age (≥10 years).

In the study of Gormally et al., GM occurred equally in both genders in the pediatric age group (17). In another study, it is more common in men (10). The prevalence of biopsy-proven CD is reported to be 1.5 times higher in women than in men (18). In our study, 53.4% of CD patients were female. GM was observed more frequently in girls in celiac patients. In females, focal morphology was observed in 13 (37.1%) cases and diffuse morphology in 32 cases (72.7%), and there was a statistically significant difference between them.

Histological findings of CD may overlap with other findings of duodenitis, and the presence of a significant neutrophilic infiltration in the duodenum often requires consideration of alternative diagnoses. Duodenal neutrophilia may develop in many diseases (such as activated non-specific chronic duodenitis, eosinophilic duodenitis, and Crohn's disease) (19). Therefore, we tried to select cases without *H. Pylori* infection or concomitant disease while forming the study group.

Case series with neutrophilia observed in CD patients are very few. It has not been conclusively determined whether CD patients have abundant neutrophils in the duodenum. Mubarak et al. compared lymphoplasmacytic, eosinophilic, and neutrophilic cell increase and gastric metaplasia in celiac and non-celiac patients, these parameters were found to be statistically significantly higher in celiac patients (20). Moran et al. observed duodenal neutrophilia frequently in celiac patients in pediatric and adult populations. In this study,

the NAS and DAS scores were 2.3 and 7.5 in the pediatric patient group and 1.2 and 8.5 in the adult population, respectively. A high NAS score was correlated with more severe histologic findings and gastric mucosa involvement. These findings suggest that the presence of neutrophils is a sign of a more active inflammatory response. In addition, a positive correlation was determined between NAS and DAS, eosinophilic infiltration, and GM in the entire cohort. In the pediatric group, there was a positive correlation between NAS and DAS and eosinophilic infiltration, but no correlation was observed between GM (2). The NAS and DAS scores were 2 and 7.5 in our study, respectively. We demonstrated a significant relationship between the development of diffuse-type GM and the increase in NAS and DAS. In addition, the diffuse-type GM, age (≥10 years), and NAS were correlated in multiple regression analysis results.

Moran et al. reported a negative correlation between NAS scores and age (2). In our study, a positive correlation was observed between GM and age. When the degree of villous atrophy was compared with GM, there was no statistically significant difference. However, the rate of diffuse-type GM was higher in celiac patients and was more common than in the control group.

In conclusion, our study considered that duodenal neutrophilia and increased disease activity might play a role in the development of GM in celiac patients. We think that it would be appropriate to include diffuse-type GM, which can be easily detected by immunohistochemical or histochemical methods, in pathology reports as a finding of histologically active CD.

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Ethics

Ethics Committee Approval: The study was approved by Eskişehir Osmangazi University Ethical Committee (Number: 2021-06, Date:25.05.2021).

This study was presented as an oral presentation at the 30th National Pathology Congress (online) on 20-23 May 2021, inTurkey.

Informed Consent: The authors declared that it was not considered necessary to get consent from the patients because the study was a retrospective data analysis.

Authorship Contributions: Surgical and Medical Practices: FC, DO, YA, ZB. Concept: FC, DO. Design: FC, DO. Data Collection or Processing: FC, DA, EY, YA, ZB. Analysis or Interpretation: FC, DO, HÖ. Literature Search: FC, DO, YA, ZB. Writing: FC, DO.

Copyright Transfer Form: Copyright Transfer Form was signed by all authors.

Peer-review: Internally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: There is no financial resource in this study