Original article

DOI: 10.5633/amm.2024.0308

MORPHOMETRIC ANALYSIS OF DUODENAL BIOPSIS IN PATIENTS WITH SUSPECTED COELIAC DISEASE

Milica Stanković[1,2], Ivan Ilić[1,3], Ivan Jovanović[4]†, Nikola Stojanović[5], Slađana Ugrenović[4], Aleksandar Milićević[1,2], Milica Lazarević[6]

[1] University Clinical Center Niš, Center for pathology and pathological anatomy, Niš, Serbia

[2] University of Niš, Faculty of medicine, Niš, Serbia

[3] University of Niš, Faculty of medicine, Department of pathology, Niš, Serbia

[4] University of Niš, Faculty of medicine, Institute for anathomy, Niš, Serbia

[5] University of Niš, Faculty of medicine, Department of physiology, Niš, Serbia

[6] University of Niš, Faculty of medicine, Department of histology, Serbia

Contact: Milica Stanković

48 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia

E-mail: stankovic.milica93@gmail.com

Coeliac disease (CD) is an immunity-mediated systemic disorder mostly presented in a form of small intestine enteropathy caused by the gluten and related prolamins intake, from cereals such as wheat, barley, and rye. The diagnosis of CD is currently based on clinical presentation, pathohistological evaluation of the small intestine biopsis and positive serology. The aim of our study was to investigate histological abnormalities in villous architecture of duodenal bulb and postbulb segment in patients diagnosed with coeliac disease and those biopsis sent for examination but this diagnosis was not made. Morphometric analysis was performed on 35 duodenal samples obtained from patients with the initial clinical diagnose of CD while some patients had dyspepsia as a primary diagnose. The obtained data of villus width measured in bulbar and postbulbar part of duodenum were found to be statistically significantly different ($p=0.0226$). Duodenal villi width in the bulbar part were significantly thicker than the ones in the postbulbar part, while value of the villous height from examinated places was not statistically significant. Also, none of cases from this study showed any extensive abnormalities in villous architecture. Beside pathohistological examination which remains a gold standard in diagnosing, morphometric analysis may also be helpful in detection of the latent forms of these entity. Having in mind the chronic persistence of this disease may indicate varies systemic disfunction, long term follow-up of these patients is necessary.

Key words: morphometry, duodenum, duodenal biopsis, coeliac disease

Originalni rad

DOI: 10.5633/amm.2024.0308

MORFOMETRIJSKA ANALIZA DUODENALNIH BIOPSIJA KOD BOLESNIKA SUSPEKTNIH NA CELIJAČNU BOLEST

Milica Stanković [1,2], Ivan Ilić [1,3], Ivan Jovanović [4†], Nikola Stojanović [5], Slađana Ugrenović [4] , Aleksandar Milićević [1,2], Milica Lazarević [6]

[1] Univerzitetski klinički centar Niš, Centar za patologiju i patološku anatomiju, Srbija

[2] Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

[3] Univerzitet u Nišu, Medicinski fakultet, Katedra za patologiju, Niš, Srbija

[4] Univerzitet u Nišu, Medicinski fakultet, Institut za anatomiju, Niš, Srbija

[5] Univerzitet u Nišu, Medicinski fakultet, Katedra za fiziologiju, Niš, Srbija

[6] Univerzitet u Nišu, Medicinski fakultet, Katedra za histologiju, Niš, Srbija

Kontakt: Milica Stanković

Bulevar dr Zorana Đinđića 48, 18000 Niš, Serbia

E-mail: stankovic.milica93@gmail.com

Celijačna bolest je imunološki posredovano sistemsko oboljenje najčešće prezentovano u vidu enteropatije tankog creva izazvane unošenjem glutena i njemu sličnih prolamina iz žitarica poput pšenice, ovasa i raži. Dijagnoza celijačne bolesti je aktuelno zasnovana na kliničkoj prezentaciji, patohistološkoj analizi biopsija tankog creva i pozitivnoj serologiji. Cilj našeg rada bio je da utvrdimo histološke promene u strukturi resica bulbusa i postbulbarnog dela duodenuma kod bolesnika sa dijagnozom celijačne bolesti i onih kod koje ista nije utvrđena. Morfometrijska analiza je sprovedena na 35 duodenalnih uzoraka dobijenih od bolesnika sa uputnom dijagnozom celijakije, dok su neki imali dispepsiju kao primarnu dijagnozu. Dobijeni rezultati o širini resica merenoj u bulbusu i postbulbarnom delu bili su statistički značajni (p=0.226). Širina resica u bulbusu duodenuma bila je značajno veća od onih u postbulbarnom delu, dok vrednost visine resica na ispitivanim mestima nije bila statistički značajna. Takođe, nijedan slučaj u ovoj studiji nije pokazao značajne promene u vilusnoj građi. Pored patohistološke analize koja predstavlja zlatni standard u dijagnostici, morfometrijska analiza može takođe biti od pomoći u otkrivanju latentnih formi ovog entiteta. Imajući u vidu da hronično perzistiranje ove bolesti može usloviti brojne sistemske poremećaje, dugoročno praćenje ovih bolesnika je neophodno.

Ključne reči: morfometrija, duodenum, duodenalne biopsije, celijačna bolest

MORPHOMETRIC ANALYSIS OF DUODENAL BIOPSIS IN PATIENTS WITH SUSPECTED COELIAC DISEASE

Introduction

Coeliac disease (CD) is an immunity-mediated systemic disorder mostly presented in a form of small intestine enteropathy caused by the gluten and related prolamins intake, from cereals such as wheat, barley, and rye (1). Clinical presentation of CD varies, but it is mostly characterized by a combination of gastrointestinal symptoms, such as malabsorption, persistant diarrhea, abdominal discomfort, pain, and extra-intestinal manifestations, which include dermatitis herpetiformis, nutritional deficiency, anemia, osteoporosis, endocrine and neurologic disorders (2). However, some patients may be asymptomatic or have discrete signs of the disease (3). The pathogenesis of this intestine injury is presented as an interaction between inflammatory cells (IELs) from the lamina propria and gliadin from food sources (4). The diagnosis of CD is currently based on clinical presentation, pathohistological evaluation of the small intestine biopsies and positive serology. In some clinical cases, the diagnostic criteria can be ambiguous, so a precise evaluation of the laboratory and histopathological results is necessary (5).

Mostly, this autoimmune disease primarily affects the superficial mucosa of the small intestine, while deeper layers are rarely implicated (5,6). Thus, the histologic examination of mucosal changes might be considered as a gold standard for CD diagnosis, since it is present in patients both with/without clinical symptoms or signs (7). The most characteristic histological features of CD are abnormalities in villous architecture with a reduction in villus height (Vh), a crypt hyperplasia with an increase in its depth (Cd), and inflammatory cell infiltration, which mainly comprises of IELs (7,8). It is also known as a condition characterized by normal villous structure with discreet increase in the number of inflammatory cells and crypt hyperplasia, defined as "microscopic enteritis" (9). The pathohistologic diagnose of CD is mainly based on Marsh-Oberhuber semiquantitative classification which grades the small intestine changes into four categories, with several subgrades, depending on the specific changes (10). The disturbance in the normal villous architecture are found to be the features of the type 3 and 4 presented as a different degree of villous blunting, flattening, or as a hypoplastic lesions, while type 1 and 2 show alterations only in number of the IELs, without any histological abnormalities (6,11).

As a result of the higher levels of acid in the duodenal lumen, mucosal morphology is characterized with short or broad villi, sometimes branching, while in the lamina propria greater number of inflammatory cells are present (12,13). On the other hand, patients with active and untreated CD often have various changes in the mucosal architecture, such as villous atrophy (VA), crypt elongation, flattening of the surface epithelium, decrease the number of Goblet cells and increase of the lymphocytes and plasmocytes in the epithelium of the villi and crypt, and also in the lamina propria (13,14). Interestingly, these histological abnormalities aren't usually only present in the patients with CD, but also could be found in a variety of disorders including inflammatory bowel disease (Crohn disease), autoimmunity or immunodeficiency, infection, nutritional and medication-related disorders (15).

Mucosal changes in patients with suspected CD are mostly presented in duodenal bulb, and the biopsy samples taken from there may be useful in diagnosing this disorder (14,16). Also, histological examination of the differences between biopsy obtained from duodenal bulb and the second part of the duodenum may help in interpretation of the intestinal abnormalities in these specific entity (13,14).

The aim of our study was to investigate histological abnormalities in villous architecture of duodenal bulb and postbulb segment in patients with suspected CD.

Material and methods

The morphometric analysis was performed on 35 duodenal samples obtained from patients, aging from 18 to 30, by routine endoscopic procedure. Analyzed duodenal specimens are part of the collection database of the Centar for pathology and pathological anatomy, University Clinical Center Niš, Serbia. Duodenal samples were routinely processed and stained with hematoxylin and eosin (H&E) following the standard protocol. Biopsies were examined using a light microscope Olympus BX50 (Olympus, Japan) connected with a digital camera Leica DFC 295 (Leica Microsystems, Germany) at the Morphometric laboratory, Department of Anatomy, Faculty of Medicine, University of Niš.

In most cases, the initial clinical diagnose was coeliac disease, while some patients had dyspepsia as a primary diagnose. From each patient the duodenal mucosa sample was obtained from both duodenal bulb and postbulbar segment of the duodenum. Five high magnification fields (×200) from each specimen were photographed, and non-processed images analyzed in

the ImageJ (http://rsb.info.nih.gov/ij/) software. Examined morphometric parameters included villus length and width of the bulbar and postbulbar duodenum part expressed in μm. Villus height was measured from base of the villi to its basal lamina, not taking the epithelial surface into account. In the case of the villous width it was expressed as the mean value obtained after the measurement of width in the base, middle and apical part of the villous (Figure 1).

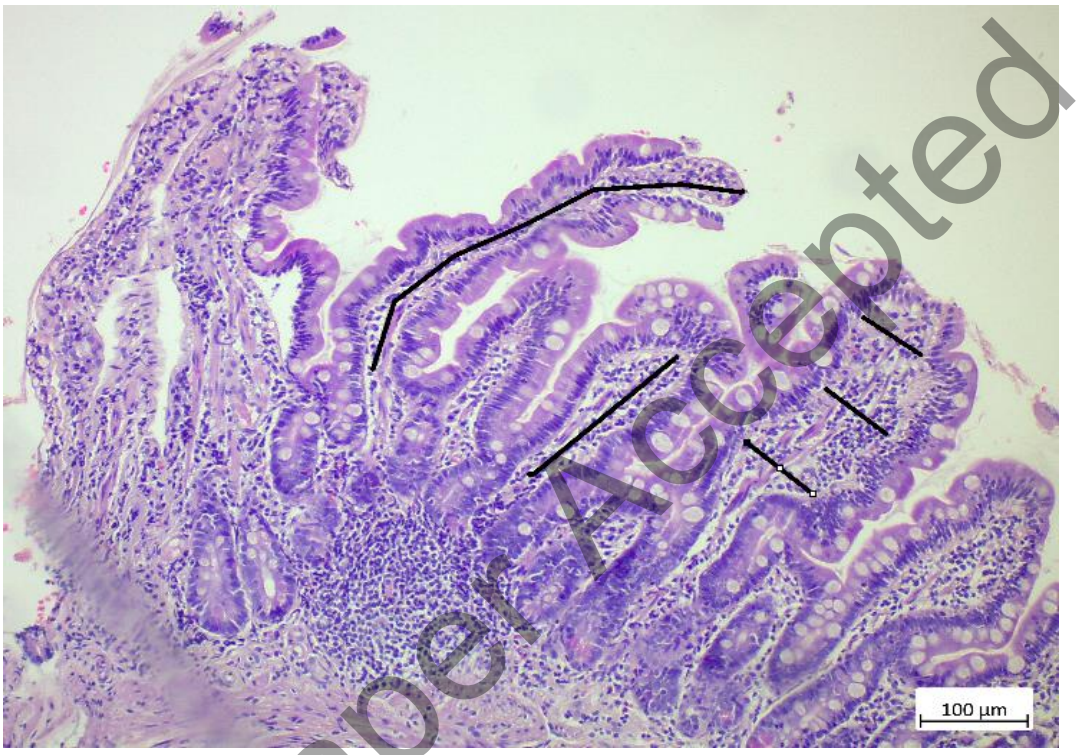

Figure 1. Example of morphometric measurement of the villus height and villus width in duodenal bulb (H&E, magnification ×100)

Statistical analysis

The obtained data are given as mean ± SD and further compared using Student's t-test (GraphPad Prism, 8.0). Probability values ($p) \leq 0.05$ were considered to be statistically significant.

Results

In 35 examinated cases, the value of villus height obtained from bulbar part of the duodenum ranged from 145 to 365 μm (Figure 1). On the other hand, the same morphometric parameter measured in the second part of the duodenum (postbulbar) showed values ranging from 166 to

322 μm. When the villus height in the two measured parts was compared no statistically significant differences were found (Figure 2).

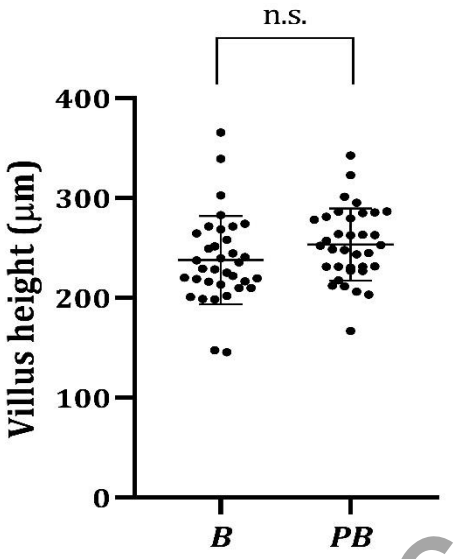

Figure 2. Villus height in bulbar and postbulbar part of the duodenum, n.s. – no statistically significant difference found using Student's t test

statistically significant difference found using Student's t test (p=0.0226)

The obtained data of villus width measured in bulbar and postbulbar part of duodenum were found to be statistically significantly different (p=0.0226) (Figure 3). Duodenal villi width in the bulbar part were significantly thicker (mean value 47.6 μm) than the ones in the postbulbar part (mean value 43.7 μm).

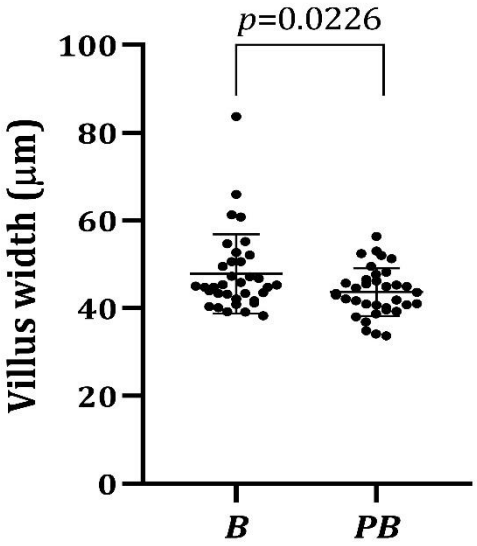

Figure 3. Villus width in bulbar and postbulbar part of the duodenum with

None of the examined cases in this study showed any extensive abnormalities in villous architecture. In the most cases normal villous morphology, without destructive lesions were observed (Figure 4). Based on these findings, our patients could be categorized as lower grade according to the Marsh-Oberhuber classification.

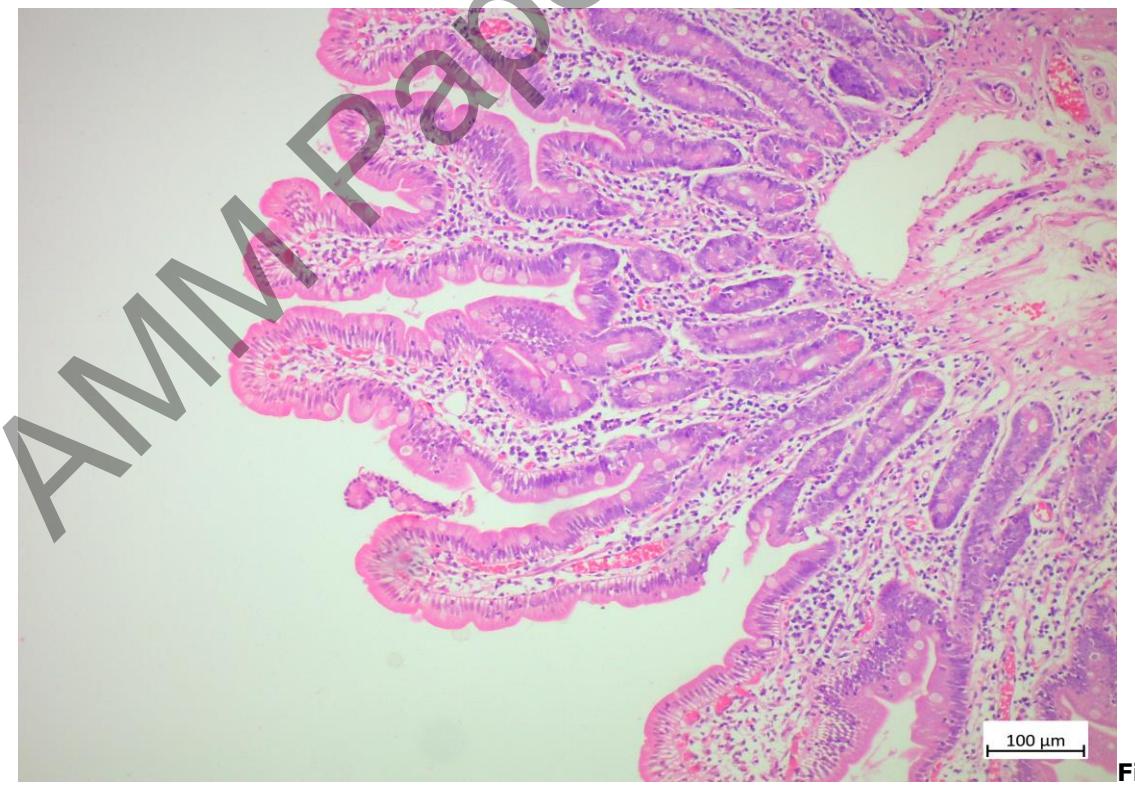

Figure 4. Pathohistological examination of biopsy obtained from the duodenal bulb of patient

suspected to coeliac disease, showing the normal villous architecture with discreet increase of the number of IELs (H&E, magnification ×100).

Discussion

The diagnosis of the CD, a complex autoimmune disorder, is based on clinical and histological findings, as well as on the positive serology (16). Knowing that higher levels of the transglutaminase antibodies may suggest presence of the coeliac disease, in case of seronegative patients with evidential clinical signs, the most clinicians assert the necessity of the histological examination (16,17). The most frequent clinical symptom seen in patients with CD is bloating which is often accompanied with either diarrhea, constipation, heartburn or nutritional deficiency (8,18). All studied patients presented similar gastrointestinal symptoms, however, no additional information were given about some extraintestinal disorders. In routine clinical practice, some disorders may imitate CD such as *Helicobacter pylori* infection, giardiasis, autoimmune enteropathy, eosinophilic gastroenteritis, drug-induced enteropathy, intestinal lymphoma, Crohn's disease, tropical sprue, etc. (18,19). Moreover, the diagnosis of CD should be clearly separated from that of gastroduodenal inflammation (gastroduodenitis), which has almost identical clinical symptomatology, but with no significant mucosal disturbances (20).

Distal duodenum and proximal jejunum represent the best sites for detecting villous abnormalities which are seen in CD (21). In most patients, the degree of the VA was present especially in distal part of the duodenum, while some of them did not have any abnormalities at other examined locations (19). Thus, it is suggested that the most representative sampling site in patients suspected for CD is duodenal bulb and distal duodenum, from where two and four biopsies, respectively, should be taken and compared (9). The design of this study overlaps with a previous one (11), where the comparison of the two duodenal segments was shown to have a significant rational. Some authors suggest that beside adequate number of biopsies, the orientation of a sample, in position 9 and 12 o'clock, is necessary for precise evaluation of the degree of VA (12,16,22). Furthermore, it is desirable to cut biopsy samples at a right angle, where mucosa and crypt must be cut longitudinally in order to obtain a better image(s) for morphometric measurements (22,23).

Duodenal biopsis obtained from the patients suspected with CD, atypical, asymptomatic or subclinical manifestation, may exert various grades of VA, often with typical endoscopic features such as "mosaic", "scalloping" or flattening of duodenal folds and emphasized vascular patterns (6,18). Also, the characteristic mucosal changes in patients with CD are mostly presented with abnormalities in villous architecture and a reduction in Vh, crypt hyperplasia with an increase in its Cd, and inflammatory cell infiltration, especially of the IELs (9,10,15). Furthermore, study conducted by Chaudhari et al. suggest various forms of villous lesions from flattening to the atrophy with moderate density of inflammatory cells and duodenal metaplasia (24).

Here studied biopsies were taken following mentioned recommendations and the results imply significantly larger villous width in the bulbar part of duodenum, than in the post-bulbar (Fig 3). These findings are in accordance with some previous ones (9), however, no significant deviation in villus height was noted as stated elsewhere (11,14). Furthermore, examination of the duodenal bulb villi showed possibility of its shortening, blunting and sometimes absence of the Brunner's glands and lymphoid aggregates, which can be the result of higher secretion of gastric acid (23,25).

Compared to the normal intestinal samples, inflamed duodenal mucosa show broader villi above the Brunner's glands while significant difference in villus length wasn't confirmed by our investigation, nor was that found in other studies (23). Significant villous width may be explained by the dilatation of the Brunner's glands, extensive inflammation and lympho-plasmocyte infiltration of the lamina muscularis mucose and sometimes gastric metaplasia of the duodenal epithelium (11).

Interestingly, in some cases mucosal changes may be absent or minimal, beside representative clinical symptoms and positive serology (25). Similar observation was noticed in many here-studied cases. Some authors suggest that measurement of morphometric parameter defined as ratio between villus height and crypt depth (Vh:CrD ratio) can be helpful in detecting latent and minimal mucosal lesions, with a potential of taking the second duodenal biopsy for long term follow-up of these patients (21,26,27). It is worth mentioning that pathohistological examination of the biopsy samples of patients undergoing gluten free diet also represent a significant challenge for pathologist because in that case mucosal changes may disappear (28).

Conclusion

Coeliac disease, as a complex inflammatory condition that affects multiple organ systems, has a possibility for many nonmalignant and malignant complications. Since the diagnosing is based on the correlation between clinical presentation, histologic features and positive serology, pathohisthological examination of the small intestine remains a gold standard. Detailed morphometric analysis of the mucosal changes could help detect latent forms of this gluten mediated disorder. Based on the findings of our study villi width was significantly higher in duodenal bulb than in postbulbar part, while the villous height was unaltered, suggesting a slight changes occurring in some borderline cases. These results could be obtained only if several biopsies taken from two anatomical sites are analyzed, which implies that it should be a routine practice in the diagnosis of coeliac disease.

Acknowledgements

The authors dedicate this paper to deceased professor Ivan Jovanović (MD, PhD), as a gratitude for the unreserved support, motivation, and selfless share of his knowledge.

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