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MORPHOMETRIC ANALYSIS OF DUODENAL BIOPSIES IN PATIENTS WITH SUSPECTED COELIAC DISEASE

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Coeliac disease (CD) is an immune-mediated systemic disorder mostly presented in the form of small intestine enteropathy caused by 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 biopsies and positive serology. The aim of our study was to investigate histological abnormalities in the villous architecture of the duodenal bulb and postbulb segment in patients diagnosed with CD and in those biopsies sent for examination but the diagnosis was not confirmed. Morphometric analysis was performed on 35 duodenal samples obtained from patients with the initial clinical diagnosis of CD while some patients had dyspepsia as a primary diagnosis. The obtained data of villus width measured in the bulbar and postbulbar part of the duodenum were found to be statistically significantly different ($p = 0.0226$). The width of the duodenal villi in the bulbar part was significantly thicker than the one in the postbulbar part, while the value of the villous height at the examined places was not statistically significant. Also, none of the cases in this study showed any extensive abnormalities in villous architecture. Besides pathohistological examination which remains the gold standard in diagnosing, morphometric analysis may also be helpful in the detection of the latent forms of this entity. Having in mind that the chronic persistence of this disease may indicate various systemic dysfunction, long-term follow-up of these patients is necessary.

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Key words: *morphometry, duodenum, duodenal biopsies, coeliac disease*

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Introduction

Coeliac disease (CD) is an immune-mediated systemic disorder mostly presented in the form of small intestine enteropathy caused by 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, persistent diarrhoea, abdominal discomfort, pain, and extra-intestinal manifestations, which include dermatitis herpetiformis, nutritional deficiency, anaemia, 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 the 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), crypt hyperplasia with an increase in its depth (Cd), and inflammatory cell infiltration, which mainly comprises IELs (7, 8). It is also known as a condition characterized by a normal villous structure with a discreet increase in the number of inflammatory cells and crypt hyperplasia, defined as "microscopic enteritis" (9). The pathohistological diagnosis of CD is mainly based on the Marsh–Oberhuber semiquantitative classification which grades the small intestine changes into four categories, with several subgrades, depending on the specific changes (10). The disturbances in the normal villous architecture are found to be the features of the types 3 and 4 presented as a different degree of villous blunting, flattening, or a hypoplastic lesion, while types 1 and 2 show alterations only in the 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 by short or broad villi, sometimes branching, while in the lamina propria, a 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 in 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 are not usually present only in patients with CD, but also could be found in a variety of disorders including inflammatory bowel disease (Crohn's disease), autoimmune or immunodeficiency infection, nutritional and medication-related disorders (15).

Mucosal changes in patients with suspected CD mostly present in the 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 the biopsy obtained from the duodenal bulb and the second part of the duodenum may help in the interpretation of the intestinal abnormalities in this specific entity (13, 14).

The aim of our study was to investigate histological abnormalities in the villous architecture of the 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 aged from 18 to 30, by routine endoscopic procedure. Analyzed duodenal specimens are part of the collection database of the Centre for Pathology and Pathological Anatomy, University Clinical Centre 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 CD, 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 the 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).

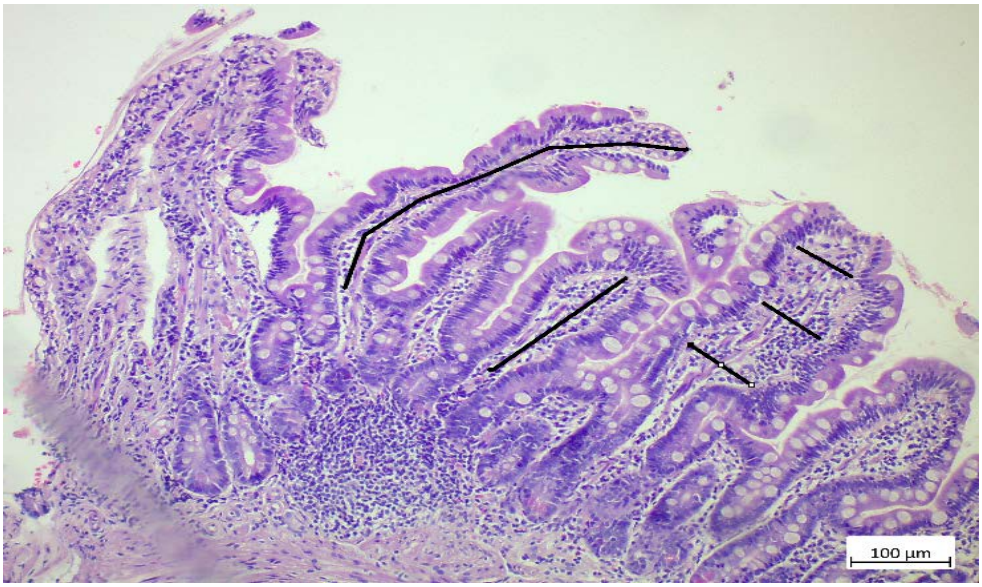

Figure 1. Example of morphometric measurement of the villus height and width in the duodenal bulb (H&E, magnification ×100)

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) ≤ 0.05 were considered to be statistically significant.

Results

In 35 examined 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 of the two measured parts was compared, no statistically significant differences were found (Figure 2).

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

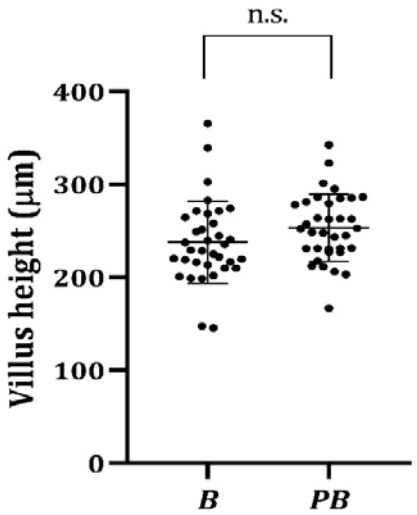

Figure 2. Villus height in the bulbar and postbulbar part of the duodenum, ns—no statistically significant difference was found using the Student's t-test

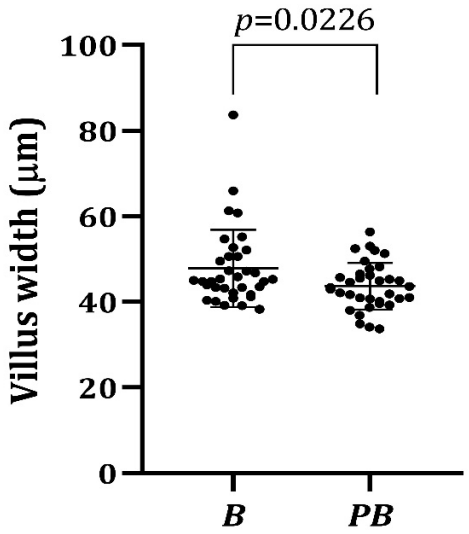

Figure 3. Villus width in the bulbar and postbulbar part of the duodenum with a statistically significant difference of p = 0.0226 was found using the Student's t-test

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

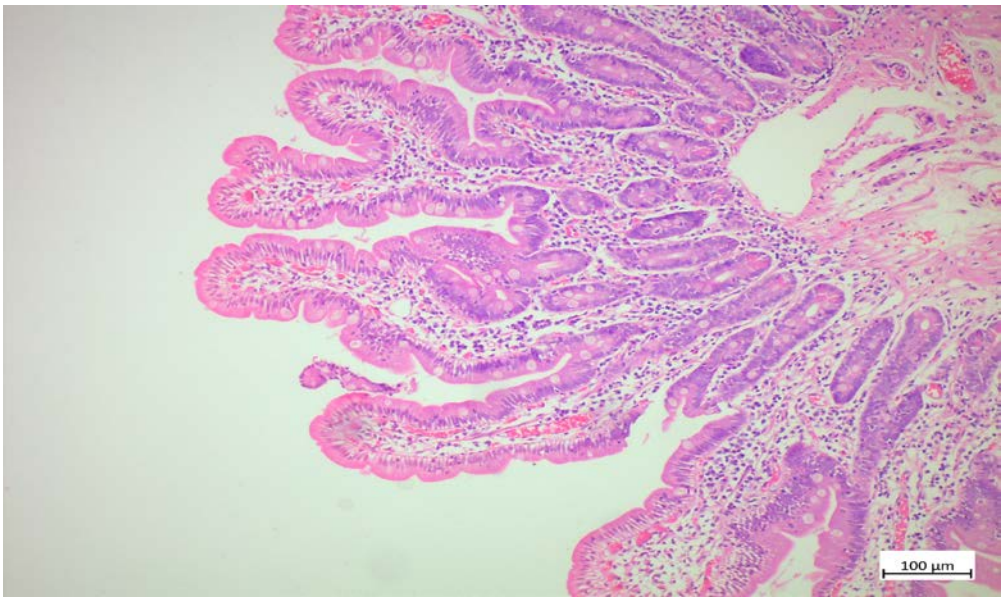

Figure 4. Pathohistological examination of a biopsy obtained from the duodenal bulb of the patient suspected of coeliac disease, showed normal villous architecture with a discreet increase in the number of IELs (H&E, magnification ×100)

Discussion

The diagnosis of CD, a complex autoimmune disorder, is based on clinical and histological findings, as well as on positive serology (16). Knowing that higher levels of transglutaminase antibodies may suggest the presence of CD, in the case of seronegative patients with evident clinical signs, 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 diarrhoea, constipation, heartburn or nutritional deficiency (8, 18). All studied patients presented with similar gastrointestinal symptoms, however, no additional information was 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 VA was present especially in the 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 sites in patients suspected of CD are the 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 rationale. Some authors suggest that besides the 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 the right angle, where mucosa and crypt must be cut longitudinally in order to obtain a better image(s) for morphometric measurements (22, 23).

Duodenal biopsies obtained from patients suspected of having 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, a study conducted by Chaudhari et al. suggested various forms of villous lesions from flattening to atrophy, with a moderate density of inflammatory cells and duodenal metaplasia (24).

In this study, investigated biopsies were taken following the mentioned recommendations and the results implied significantly larger villous width in the bulbar part of the duodenum, than in the post-bulbar (Figure 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 a possibility of their shortening, blunting and sometimes the absence of 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 shows broader villi above Brunner's glands while a significant difference in villus length was not confirmed by our investigation, which is consistent with other studies (23). Significant villous width may be explained by the dilatation of Brunner's glands, extensive inflammation and lymph-plasmocyte infiltration of the lamina muscularis mucosae and sometimes gastric metaplasia of the duodenal epithelium (11).

Interestingly, in some cases, mucosal changes may be absent or minimal, besides representative clinical symptoms and positive serology (25). A similar observation was noticed in many here-studied cases. Some authors suggest that measurement of the morphometric parameter defined as the 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 represents a significant challenge for pathologists because in that case, mucosal changes may disappear (28).

Conclusion

Coeliac disease, as a complex inflammatory condition that affects multiple organ systems, provides a possibility for many nonmalignant and malignant complications. Since the diagnosis is based on the correlation between clinical presentation, histologic features and positive serology, pathohistological examination of the small intestine remains the 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 the duodenal bulb than in the postbulbar part, while the villous height was unaltered, suggesting that slight changes occurred in some borderline cases. These results could be obtained only if several biopsies taken from two anatomical sites were analyzed, which implies that it should be a routine practice in the diagnosis of coeliac disease.

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MORFOMETRIJSKA ANALIZA BIOPSIJA DUODENUMA KOD BOLESNIKA KOD KOJIH POSTOJI SUMNJA NA POSTOJANJE CELIJAČNE BOLESTI

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Celijačna bolest je imunološki posredovano sistemsko oboljenje koje se najčešće prezentuje u vidu enteropatije tankog creva izazvane unošenjem glutena i njemu sličnih prolamina iz žitarica poput pšenice, ječma i raži. Dijagnoza celijačne bolesti je trenutno 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 kod osoba kod kojih ona nije utvrđena. Morfometrijska analiza sprovedena je na 35 duodenalnih uzoraka dobijenih od bolesnika kod kojih postoji sumnja na postojanje celijakije, dok su neki od bolesnika imali dispepsiju kao primarnu dijagnozu. Dobijeni rezultati o širini resica merenih u bulbusu i postbulbarnom delu bili su statistički značajni ($p = 0,226$). Širina resica u bulbusu duodenuma bila je značajno veća od širine resica 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 građi vilusa. Pored patohistološke analize, koja predstavlja zlatni standard u dijagnostici, morfometrijska analiza takođe može biti od pomoći u otkrivanju latentnih formi ove pojave. S obzirom na to da hronično perzistiranje ove bolesti može usloviti brojne sistemske poremećaje, neophodno je dugoročno praćenje ovih bolesnika.

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***Ključne reči**: morfometrija, duodenum, biopsije duodenuma, celijačna bolest*