

Concentrations of antiganglioside M1 antibodies, neuron-specific enolase, and interleukin 10 as potential markers of autonomic nervous system impairment in celiac disease

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KEY WORDS

antiganglioside M1 antibodies, celiac disease, interleukin 10, neuron-specific enolase

ABSTRACT

INTRODUCTION Celiac disease (CD) is an immune-mediated enteropathy related to permanent gluten intolerance, characterized by gastrointestinal symptoms as well as nongastrointestinal symptoms, including neurologic ones. The presence of neuron-specific enolase (NSE), interleukin 10 (IL-10), and antiganglioside M1 (anti-GM1) antibodies has been demonstrated for neurologic conditions as well as immune disorders with neurologic manifestations.

OBJECTIVES The aim of the study was to determine the concentrations of IL-10, NSE, and anti-GM1 antibodies in the course of CD and their correlation with changes in electrogastrography (EGG) and with heart rate variability (HRV).

PATIENTS AND METHODS The study included 68 participants: 34 patients with CD and 34 healthy individuals. We assessed the concentrations of IL-10 and NSE as well as the presence of anti-GM1 antibodies in serum. We investigated correlations between the concentrations of IL-10, NSE, and anti-GM1 antibodies and the results of EGG and HRV.

RESULTS Patients with CD had a higher level of anti-GM1 antibodies than controls (1.38 ng/ml [0.98–2.03 ng/ml] vs 0.81 ng/ml [0.35–1.15 ng/ml]). Median IL-10 concentrations in patients with CD differed significantly from those in controls (7 pg/ml [4.33–11.48 pg/ml] vs 4.27 pg/ml [2.44–7 pg/ml]; $P = 0.010$). In HRV analysis, a positive correlation between IL-10 concentrations and very low frequency spectrum was observed ($r = 0.63$; $P = 0.003$). There was no correlation between the concentrations of IL-10, NSE, or anti-GM1 antibodies and EGG parameters.

CONCLUSIONS Chronic inflammation in the course of CD may lead to autonomic nervous system impairment and development of neurologic disorders. Therefore, anti-GM1 antibodies and IL-10 may be considered as markers of nervous system impairment in the course of CD.

INTRODUCTION Celiac disease (CD) is a chronic inflammatory enteropathic disease triggered in genetically susceptible individuals by the ingestion of grain (wheat, barley, rye) containing

proteins rich in proline and glutamine. As a result of the activation of the innate and adaptive immune system, histological lesions such as intraepithelial lymphocytosis, crypt hypertrophy,

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TABLE 1 Gastrointestinal, nonintestinal, and neurological symptoms of celiac disease

Gastrointestinal symptoms	Nonintestinal symptoms	Neurological symptoms
chronic diarrhea	anemia	polyneuropathy
abdominal pain	elevated aminotransferase levels	autonomic nervous system disturbances
abdominal distention	dermatitis herpetiformis	brain white matter lesions
vomiting	enamel hypoplasia	cerebellar ataxia
constipation	recurrent aphthous stomatitis	epilepsy
	developmental delay short stature delayed puberty	headache
	recurrent miscarriage infertility	myoclonus

TABLE 2 Characteristics of the study groups

Parameter		Celiac disease	Control group	P value
sex, n (%)	female	27 (79.4)	23 (67.6)	0.272
	male	7 (20.6)	11 (32.54)	
age, y	n	34	34	0.351
	mean \pm SD	41.18 \pm 16.58	35.82 \pm 9.49	
	median (Q1–Q3)	36.5 (28.5–56.25)	34 (29.25–43)	
	min–max	18–70	22–54	
NSE, ng/ml	n	34	34	0.659
	mean \pm SD	5.20 \pm 8.18	3.76 \pm 1.62	
	median (Q1–Q3)	3.44 (2.98–4.13)	3.44 (3.292–4.06)	
	min–max	2.33–50.76	1.74–9.24	
anti-GM1 antibodies, ng/ml	n	31	33	<0.001
	mean \pm SD	1.59 \pm 1.07	–0.83 \pm 0.67	
	median (Q1–Q3)	1.38 (0.98–2.03)	0.81(0.35–1.15)	
	min–max	0.06–4.82	0.0063.74	
IL-10, pg/ml	n	34	27	0.010
	mean \pm SD	8.52 \pm 6.34	7.78 \pm 16.06	
	median (Q1–Q3)	7 (4.33–11.48)	4.27 (2.44–7)	
	min–max	1.05–35.3	1.05–8.68	
EmA, n (%)	negative	18 (81.8)		
	positive	4 (18.2)		
TTG, n (%)	negative	11 (36.7)		
	positive	19 (63.3)		
Marsh, n (%)	1	4 (22.2)		
	3	14 (77.8)		

Abbreviations: anti-GM1 antibodies, antibodies against M1 gangliosides; IL-10, interleukin 10; EmA, antiendomysium antibodies; NSE, neuron specific enolase; TTG, anti-transglutaminase antibodies; Marsh, histopathological changes according to Marsh classification

and intestinal villous atrophy occur within the small intestine. The lesions lead to a reduction of the absorption surface in the small intestine.^{1,2} Inflammatory lesions in patients with active CD are related to the response to gluten and are associated with the production of proinflammatory and anti-inflammatory cytokines such as interleukin 10 (IL-10), a pleiotropic cytokine

of anti-inflammatory activity.³ One of the main tasks of IL-10 is to maintain the immune balance within the structures of the gastrointestinal tract^{4,5} and to reduce inflammation in the mechanism of autocrine signaling.³

CD is one of the most common food enteropathies. Its prevalence is 1:100 and 1:300 in the adult population of North America and Europe, respectively, and its incidence is estimated at 2–13/100 000 per year.^{6–9} The disease affects individuals at any age, but most cases are diagnosed in early childhood and between 30 and 40 years of age. Despite significant development of diagnostic methods, the number of patients suffering from CD remains underestimated. The ratio of diagnosed to undiagnosed population is 1:5–10.^{6–9}

The spectrum of clinical symptoms in the course of CD is very broad and encompasses gastrointestinal symptoms, such as diarrhea and abdominal distention, but also various other manifestations, including neurologic symptoms (TABLE 1).^{2,10–16} The first reports of concomitant neurologic disorders within the central nervous system in patients affected with CD appeared more than 40 years ago, but only in the last few years, research on the occurrence of gluten-dependent peripheral neuropathy and disorders of the autonomic nervous system (ANS) has been conducted.^{11,15,17,18}

Neurologic and psychiatric symptoms have already been observed in 22% of patients with CD.¹⁷ In their study, Isiskay et al¹⁹ found neurologic symptoms in more than 13% of children suffering from CD. Moreover, it has also been determined that more than half of the patients with neurologic symptoms of unknown etiology have positive serological markers typical of CD.²⁰ Previous studies have also shown the occurrence of ANS disorders²¹ and the simultaneous occurrence of these disorders with impairment of gastric myoelectrical activity.²²

The presence of antiganglioside antibodies in neurologic disorders has been reported.²³ A few study groups^{11,17,23} have described the presence of these antibodies in some patients with CD, including patients with no neurologic symptoms. Gangliosides constitute a heterogeneous group of acidic glycolipids. They are located on the surface of neurons and perform diverse roles in a number of reactions. They have been classified as GM1, GM2, GD1a, GD1b, GT1b, and GQ1b.²⁴ Antibodies to GM1 and GD1a seem to play a key role in demyelinating processes.²⁴

Studies have confirmed that in the course of immune disorders, abnormalities in the nervous system were accompanied by changes in the concentration of neuron-specific enolase (NSE).²⁵ NSE belongs to glycolytic enzymes and participates, among others, in the mechanisms affecting the inhibition of inflammatory response.^{26–30} In nervous system disorders, variations in the concentration of NSE were observed. Three subunits of enolase have so far been determined in higher eukaryotic representatives: alpha-enolase located

TABLE 3 Characteristics of celiac disease group with electrogastrography and heart rate variability testing

Parameter	Celiac disease (n = 21)	Control group (n = 21)	P value
sex, n (%)	female	17 (80.9)	0.572
	male	4 (19.1)	
age, y	42.18 ± 15.8	36.82 ± 8.4	0.451
	35.5 (18–68)	34 (22–54)	
normalized LF, %	46.1 ± 16.2	48.2 ± 15.8	0.659
	42.3 (18.4–75.1)	44.8 (29.3–101.0)	
normalized HF, %	53.9 ± 16.2	55.1 ± 15.1	0.456
	58.00 (24.8–81.6)	56.2 (22.1–101.0)	
VLF-HRV, ms ²	795.3 ± 411.8	599.6 ± 421.5	0.08
	332.3 (85.7–6344.1)	407.0 (101–2305)	
LF-HRV, ms ²	966.5 ± 341.5	1396.2 ± 973.4	0.001
	298.0 (32.3–11 226.0)	973.0 (101–5544.0)	
HF-HRV, ms ²	1664.2 ± 389.2	1881.6 ± 1100.9	0.02
	365.54 (43.2–21870)	1123.0 (101–6927.0)	
PSD-HRV, ms ²	3425.7 ± 1394.3	3903.1 ± 2687.8	0.02
	959.9 (161.3–36 222.6)	2715.0 (101–14 189.0)	
LF/HF ratio	1.3 ± 0.9	0.86 ± 0.67	0.04
	0.76 (0.2–3.6)	0.81 (0.35–2.15)	
DF, cpm	2.4 ± 0.6	3.0 ± 0.3	0.04
	2.3 (1.2–3.8)	3.0 (2.6–3.8)	
DP, μV ²	16 2395.9 ± 49 908.7	108 857.5 ± 63 496.8	0.04
	49 076.5 (3458–976 436)	52879.3 (8889–523 328)	
normogastria, %	50.4 ± 18.9	86.0 ± 12.3	0.001
	45.5 (23.3–90.0)	87.5 (61.3–100)	
bradygastria, %	8.9 ± 7.3	2.6 ± 2.3	0.001
	7.45 (0.98–2.03)	0.0 (0.0–10.2)	
tachygastria, %	11.1 ± 9.9	2.6 ± 3.2	0.001
	7.7 (0.98–2.03)	0.0 (0.0–11.8)	
arrhythmia, %	28.6 ± 16.8	8.5 ± 8.5	0.001
	31.35 (0–63.3)	6.4 (0.0–21.0)	
SWC, %	55.5 ± 13.8	77.4 ± 11.9	0.001
	56.1 (23.9–88.9)	74.85 (60.6–100)	

Data are presented as mean ± SD or median (min–max) unless indicated otherwise.

Abbreviations: DF, dominant frequency; DP, dominant power; EGG, electrogastrography; HRV, heart rate variability; LF, low frequency; HF, high frequency; PSD-HRV, power spectral density; SWC, single wave coupling

in the majority of cells, beta-enolase specific for muscle cells, and gamma-enolase (NSE) associated with the cells of the nervous system.^{25,26–30}

An increase in NSE concentrations is observed in nervous system trauma, stroke, and malignancy related to the nervous system, for example, neuroendocrine tumors.^{25,26–30} In the course of some chronic diseases, such as lupus with neuropsychiatric symptoms, lower NSE concentrations were observed in comparison with healthy controls.²⁵

The correlation between IL-10 concentrations and the ANS response has been demonstrated in research.^{4,5} However, NSE and IL-10 concentrations have not been analyzed in adult celiac patients presenting with no neurologic symptoms. In addition, there has been no simultaneous comparative evaluation of antiganglioside antibodies,

NSE, and IL-10 concentrations in the course of CD and the variability of cardiac rhythm and gastric myoelectrical activity.

The aim of this study was to evaluate the prevalence of anti-GM1 antibodies, NSE, and IL-10 concentrations in patients with CD without neurologic symptoms and in the control group of healthy subjects. The correlations between anti-GM1 antibodies, NSE, and IL-10 and heart rate variability (HRV) and myoelectrical activity were also investigated in the group of celiac patients.

PATIENTS AND METHODS Bioethics The protocol of the study was approved by the Local Bioethics Committee at Jagiellonian University, Kraków, Poland (decision no. KBET/148/B/2012, as of May 24, 2012). All participants received written information about the assumptions and principles of

TABLE 4 Marsh classification modified by the Working Group of the United European Gastroenterology Federation

Type	IEL/EC	Crypt hyperplasia	Villous atrophy
0 within norm	<25/100	–	–
1 infiltrative	>25/100	–	–
2 hyperplastic	>25/100	present	–
3a partial villous atrophy (<2:1)	>25/100	present	partial
3b subtotal villous atrophy	>25/100	present	subtotal
3c total villous atrophy	>25/100	present	total
4 causes other than celiac disease	<25/100	–	total

Abbreviations: EC, enterocytes; IEL, intraepithelial lymphocytes

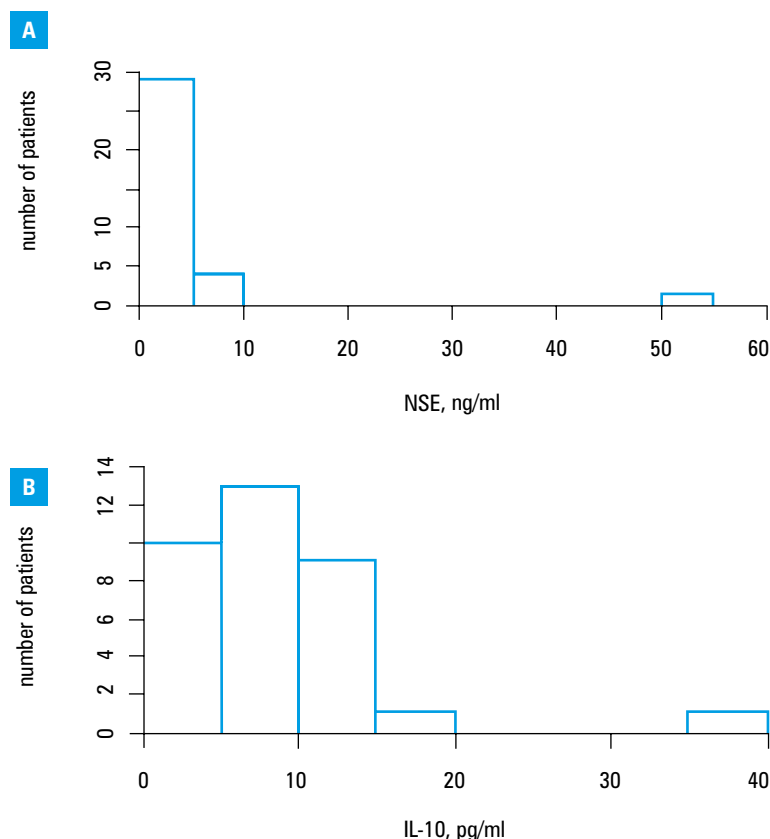


FIGURE 1 Neuron-specific enolase (NSE) (A) and interleukin 10 (IL-10) concentrations (B) in patients with celiac disease

the study and gave their informed consent to participate in the study.

Clinical examinations A total of 68 individuals were enrolled in the study, including 34 individuals with CD (50%) and 34 healthy persons (50%). Male and female percentage did not differ significantly between the celiac and healthy groups ($P = 0.272$; χ^2 test). Women constituted the majority in both groups (approx. 70% in each group). The median age of celiac patients (36.5 years [range, 28.5–56.25 years]) was not significantly different from the median age of healthy individuals (34 years [range, 29.25–43 years]) ($P = 0.351$; Mann–Whitney test). The characteristics of the examined groups are presented in TABLES 2 and 3.

Due to motion artifacts, the number of patients in the celiac group, in which EGG and HRV was performed, was reduced.

Medical history was taken and physical examination was performed in both groups. On the basis of the results, further proceedings were established and celiac patients were scheduled for a panendoscopic procedure.

Serological tests Immunoglobulin (Ig) A and IgG antiendomysial antibodies (anti-EmA) and/or antitissue transglutaminase (anti-tTG) and anti-GM1 antibodies were detected in serum. The concentrations of NSE and IL-10 were also determined. Anti-EmA and anti-tTG antibodies showed high specificity (90%–100% and 87%–97%) and sensitivity (95%–100% and 97%–100%, respectively).¹ All assays were performed according to the manufacturer's instructions.

Serum IgA EmA were detected by an immunofluorescence method. The titer above 1:10 was considered positive. Anti-tTG antibodies were assayed by an enzyme-linked immunosorbent assay (ELISA; Aesku Diagnostics GmbH, Germany). The test range was (permanently) from 0 to 300 U/ml. The concentration of antibodies equal to or higher than 15 U/ml indicated a positive result.

The serum NSE concentration was tested with an ELISA (Quantikine® ELISA Human Enolase 2/Neuron-specific Enolase Immunoassay, R&D Systems, Inc., Minneapolis, Minnesota, United States). The lowest minimum concentration (minimum detectable dose, MMD) of this assay ranged from 0.013 to 0.038 ng/ml, and the mean MMD was 0.02 ng/ml.

Anti-GM1 antibodies were detected by an ELISA test (Human Anti-ganglioside M1 Antibody ELISA Kit, ELAB, Bethesda, Maryland, United States). The assay detection range was from 3.090 to 250 ng/ml according to manufacturer's specifications.

The IL-10 concentration was determined by an ELISA test (Quantikine® ELISA Human IL-10, R&D Systems, Inc. Minneapolis, MN). The MMD was lower than 3.9 pg/ml.

Histological examination of distal duodenum mucosa biopsates

During panendoscopic biopsies, specimens were taken from the postbulbar duodenum (in accordance with current guidelines). After hematoxylin and eosin staining, the segments were assessed according to the modified Marsh classification (Oberhuber) (TABLE 4). This scale includes the density of lymphocytic infiltration, the height of villi, the depth of crypts, and the mitotic activity of the crypts. The 3a-c level is the most typical of CD.^{31,32}

Analysis of autonomic nervous system and gastric myoelectrical activity

ANS activity was recorded as the assessment of HRV at rest (Task Force Monitor 3040i, CNSystems, Graz, Austria). The myoelectric activity of the stomach (EGG)

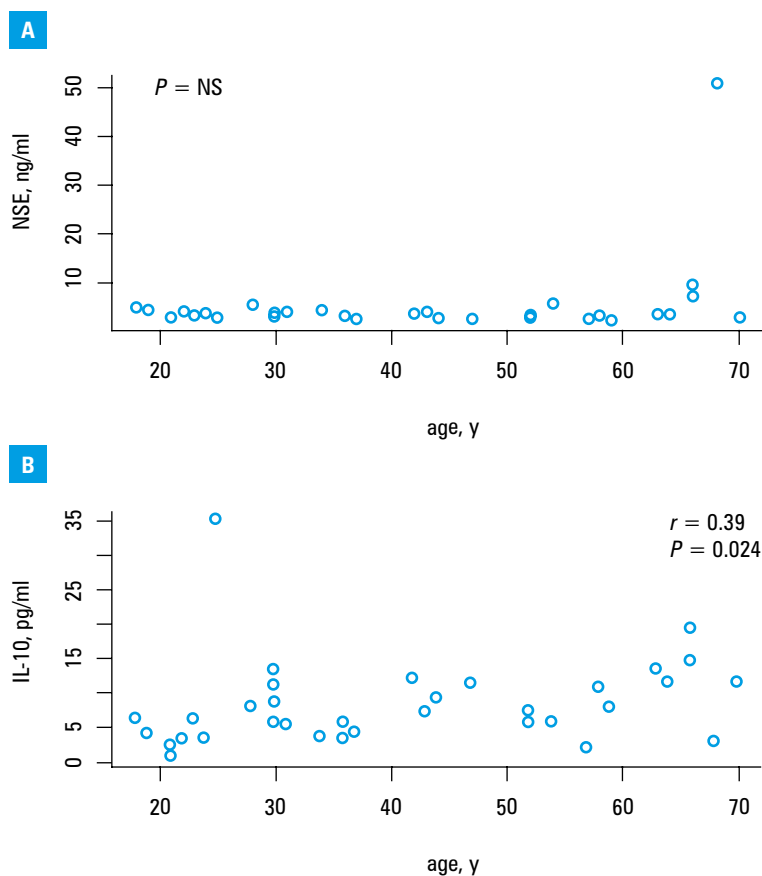


FIGURE 2 Correlations between neuron-specific enolase (NSE) concentrations and age (A) and between interleukin 10 (IL-10) concentration and age in patients with celiac disease (B). Abbreviations: NS, nonsignificant

was recorded in the fasting state simultaneously using the 4-channel electrogastrography system (Polygraf NET, Medtronic, Minneapolis, Minnesota, United States).

The following HRV components were analyzed:

- 1 a spectrum of very low frequency (VLF) range: 0.0033–0.04 Hz; it demonstrates the variability modulated by the activity of chemoreceptors and is dependent on vasomotor and thermoregulatory reflexes involving the renin–angiotensin–aldosterone system;
- 2 a spectrum of low frequency (LF) range: 0.04–0.15 Hz; it is dependent on the changes in arterial pressure and oscillation of baroreceptor reflexes and is mediated by both sympathetic and parasympathetic nerve fibers;
- 3 a spectrum of high frequency (HF) range: 0.15–0.4 Hz; it represents the effect of breathing on the heart rhythm, which depends on the modulation of the parasympathetic system;
- 4 an LF/HF ratio; it expresses the interdependence of these types of vegetative modulation and depicts the mutual relation of both autonomic nervous system components;
- 5 total power (TP); the total power of the spectrum, it reflects the activity of the entire autonomic nervous system.

EGG recordings were analyzed taking into account normogastria percentage, slow-wave coupling, and dominant power. The following exclusion criteria were introduced: concomitant neurologic disorders, malignancies, immunosuppressant treatment, medication affecting ANS activity and motility of the gastrointestinal tract, acute

and chronic viral diseases, and a history of head injury prior to the study.

Statistical analysis Correlations of anti-GM1 antibody titer, NSE, and IL-10 concentrations with disease activity measured by the modified Marsh scale (Oberhuber) and the titers of the currently used antibodies (tTG and/or EMA) were analyzed in the celiac group. The results were compared with those in the control group. The correlation between changes in EGG and HRV and the concentrations of the analyzed antibodies as well as NSE and IL-10 was assessed.

Statistical analysis was performed using Statistica 10.0 (StatSoft, Inc., Tulsa, Oklahoma, United States; the license to use the software issued for Jagiellonian University Medical College) and the free 3.2.2 version of “R” (www.r-project.org).

The level of statistical significance was set at a *P* value of less than 0.05.

RESULTS No significant effect of CD on the average results of NSE concentrations was observed ($P = 0.66$, Mann–Whitney test) (FIGURE 1). However, a significant difference between the median concentrations of anti-GM1 antibodies was noted between the groups of celiac patients and healthy controls ($P < 0.001$, the Mann–Whitney test) (FIGURE 2A and 2B). Celiac patients presented with a higher median level of anti-GM1 antibodies (1.38 ng/ml [0.98–2.03 ng/ml]) than healthy subjects (0.81 ng/ml [0.35–1.15 ng/ml]).

The median concentration of IL-10 in celiac patients (7 pg/ml [4.33–11.48 pg/ml]) differed significantly from that in the control group (4.27 pg/ml [2.44–7 pg/ml]) ($P = 0.010$; Mann–Whitney test). Celiac patients presented with higher median levels of IL-10 compared with healthy subjects.

In the celiac group, no significant correlations between age and NSE concentrations ($r = -0.02$; $P = 0.926$) or between anti-GM1 antibodies and age were demonstrated ($r = 0.15$; $P = 0.422$). A significant, positive, and strong correlation between age and IL-10 concentrations was shown ($r = 0.39$; $P = 0.024$).

In the control group, there was no significant correlation between age and NSE concentrations ($r = -0.02$; $P = 0.9$), anti-GM1 antibodies ($r = 0.18$; $P = 0.31$), and IL-10 concentrations ($r = 0.13$; $P = 0.52$).

In patients with CD, no significant correlation was shown between concentrations of NSE and anti-GM1 antibodies ($r = 0.26$; $P = 0.16$), between concentrations of NSE and IL-10 ($r = 0$, $P = 0.99$), or between concentrations of IL-10 and anti-GM1 antibodies ($r = 0.27$, $P = 0.14$) (FIGURE 3).

In the control group, a significant, positive, and semi-strong correlation between the levels of NSE and anti-GM1 antibodies was determined ($r = 0.38$; $P = 0.03$). In the control group, the correlations between NSE and IL-10 concentrations ($r = -0.16$; $P = 0.43$) and between IL-10 concentrations and anti-GM1 antibodies ($r = 0.05$, $P = 0.786$) were nonsignificant (FIGURE 4). The assessment of

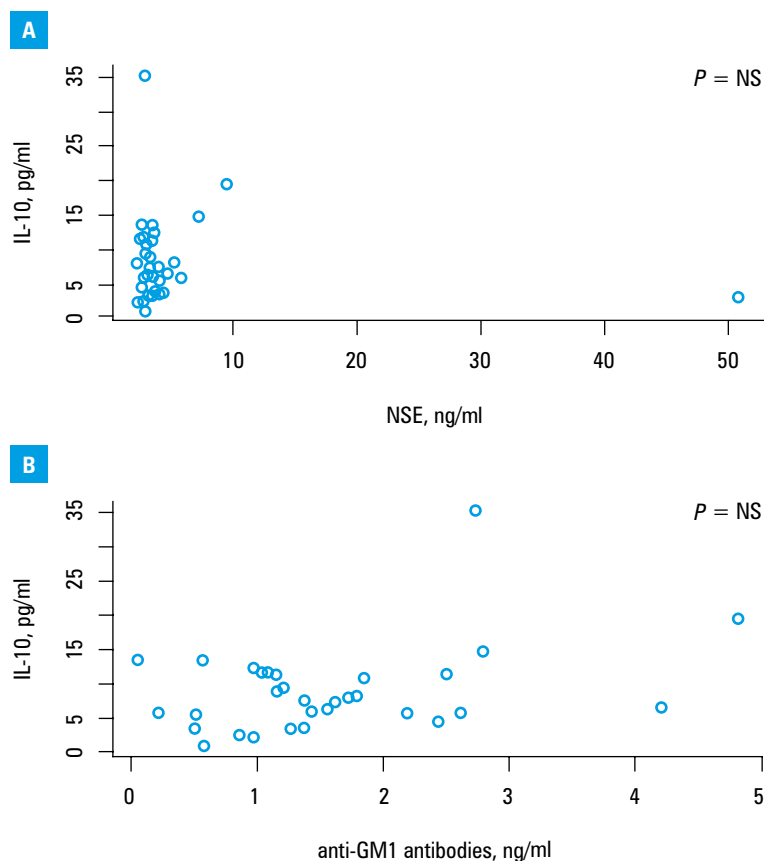


FIGURE 3 Lack of correlations between interleukin 10 (IL-10) and neuron-specific enolase (NSE) concentrations (A) and IL-10 and antibodies against M1 gangliosides (anti-GM1 antibodies) (B) in patients with celiac disease
Abbreviations: see FIGURE 1

HRV revealed a positive correlation between IL-10 concentration and VLF ($r = 0.63$; $P = 0.003$). No correlations between the concentrations of IL-10, NSE, anti-GM1 antibodies, and EGG parameters were found.

DISCUSSION The proposed pathomechanisms leading to neurologic complications imply immune responses and changes secondary to malabsorption of vitamins and microelements.^{15,31,33,34} According to Volta et al, up to 64% of patients suffering from CD with neurologic manifestation present with positive antiganglioside antibody titer.^{11,17,23} Some of the postulated mechanisms responsible for the development of neurologic symptoms in CD have been identified on the basis of studies on CD as well as gluten ataxia, which belongs to the group of gluten-dependent disorders in the spectrum of gluten sensitivity. The significance of antiganglioside antibodies demonstrated in other immune neuropathies indicates that autoimmune factors are involved in the etiopathogenesis of celiac neuropathy.^{11,17,35}

The results of our research have been confirmed by other studies. The presence of antiganglioside antibodies has so far been demonstrated in the course of gluten-dependent disorders such as CD or gluten ataxia.^{11,17,35} Allaadini et al¹¹ showed the presence of antiganglioside antibodies in 6 of 21 adult patients with CD (28%), and only 1 patient (4.7%) presented with the symptoms of peripheral polyneuropathy.¹¹ In a study on children with CD, Briani et al¹⁷ demonstrated the presence of IgM and IgG antiganglioside antibodies

in 4.8% of patients (2 cases in 42 patients) in the absence of neurologic symptoms. They revealed that the concentration of antiganglioside antibodies was not dependent on adherence to a gluten-free diet. This implies that the mechanisms for the production of these antibodies are independent of gluten intake.¹⁷

In our study group, despite the fact that all celiac patients declared compliance with dietary recommendations, histopathological changes in biopsy specimens of duodenal mucosa and/or positive results of serological tests were demonstrated in the majority of subjects. Moreover, no correlation between age and the concentration of anti-GM1 antibodies was observed. Age is considered an indirect indicator of the duration of CD. At the same time, Briani et al¹⁷ postulated that the occurrence of neurologic disorders depends on the age of patients (children vs adults).

In our study, the correlation between age and IL-10 concentrations was found in celiac patients but not in the control group. Currently, no reports regarding this type of correlation can be found in the literature.

In celiac patients, higher concentrations of IL-10 were noted, which is in contrast to the results reported by Mizrachi et al,³⁶ who observed that both the peak and total concentrations of IL-10 were significantly lower in celiac patients than in healthy subjects. The reports on the concentrations of IL-10 in patients with CD remain divergent.³⁶⁻³⁹

In our study, we demonstrated higher levels of NSE in patients with CD compared to the control group, but the difference was not significant. According to available studies, NSE concentrations in the serum of celiac patients have not been investigated. However, reports on the changes in the concentrations of NSE in the intestinal tissue in the course of CD can be found.⁴⁰

An increase in NSE expression in the mucosa of the small intestine in patients with CD and Crohn disease is indicative of enhanced neuroproliferation associated with inflammation.⁴⁰

There are no studies that evaluated the correlation between HRV parameters and IL-10 and the presence of anti-GM1 antibodies or NSE.

Our analysis of the changes in HRV recordings in patients with CD showed a significant correlation between IL-10 concentration and the VLF spectrum, while no significant correlation was noted for the remaining parameters. However, some reports concerning the correlation of IL-10 and changes in the ANS are available. In a group of people with septic shock, Papaioannou et al⁴¹ demonstrated a positive correlation between IL-10 and HF and a negative one between IL-10 and LF and IL-10 and the LF/HF ratio.

Woiciechowsky et al⁴² demonstrated that activation of the sympathetic nervous system induces the systemic release of IL-10, while blocking the parasympathetic nervous system increases inflammation in the intestine.⁴³⁻⁴⁴ In a previous study, we demonstrated the predominance

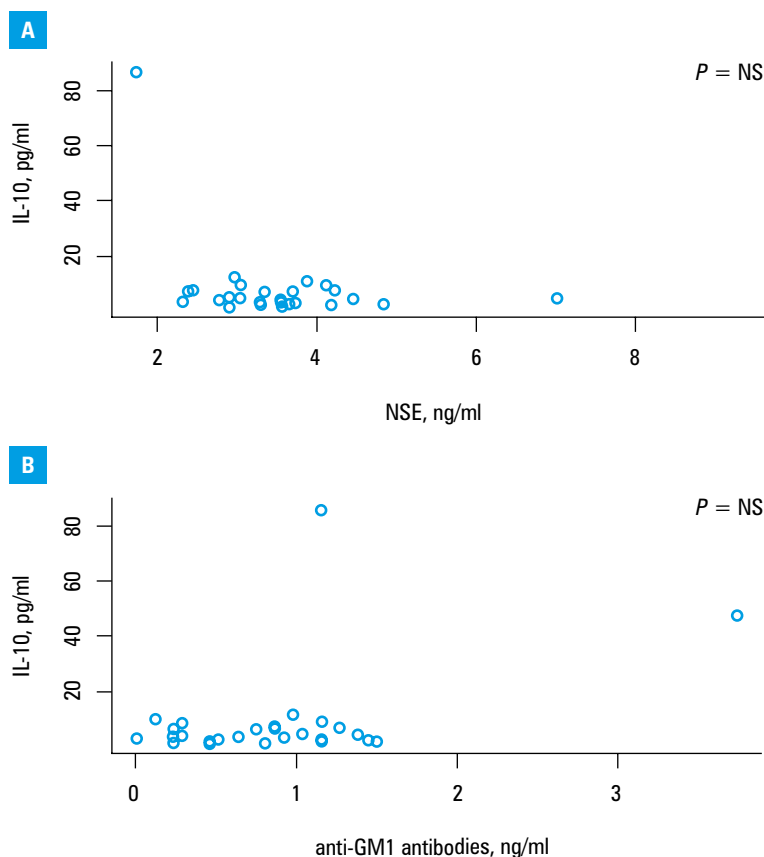


FIGURE 4 Lack of correlations between interleukin 10 (IL-10) and neuron-specific enolase (NSE) concentrations (**A**) and between IL-10 and antibodies against M1 gangliosides (anti-GM1 antibodies) in the control group (**B**) Abbreviations: see **FIGURE 2**

of the sympathetic system in patients with CD,²² which remains consistent with earlier reports.⁴²⁻⁴⁴

Gastrointestinal motility disorders accompanying CD have already been described,^{22,45,46} but the pathomechanisms inducing the disorders have not been fully clarified. One hypothesis involves changes secondary to ANS disorders.²² The correlations between the changes in gastric myoelectrical activity and the presence of antiganglioside antibodies, NSE, and IL-10 have not been analyzed yet.

Considering our current results on the correlation between IL-10 and changes in HRV recordings and our previous results, the effect of IL-10 on gastric myoelectrical activity cannot be excluded.

Owing to the pilot design of the study and the results outlined above, further studies seem warranted.

In conclusion, our study revealed that celiac patients have higher concentrations of anti-GM1 antibodies, IL-10, and NSE in comparison with the control group. The NSE level was not significantly higher in the celiac group. Moreover, changes in HRV depend on IL-10 concentrations. Finally, further studies are needed on the potential markers of ANS damage in the course of CD.

Contribution statement MP-F and MZ-W conceived the idea for the study. MP-F, AF, TM, and MZ-W contributed to the design of the research. All authors were involved in data collection. KS, AF, and MP-F analyzed the data. MP-F and MZ-W coordinated funding for the project. All authors edited and approved the final version of the manuscript

REFERENCES

- Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr.* 2012; 54: 136-160.
- Asamoah V, von Coelln R, Savitt J, Lee LA. The many faces of celiac disease. *Gastroenterol Hepatol (N Y).* 2011; 8: 549-554.
- Forsberg G, Hernell O, Hammarström S, et al. Concomitant increase of IL-10 and pro-inflammatory cytokines in intraepithelial lymphocyte subsets in celiac disease. *Int Immunol.* 2007; 19: 993-1001.
- Girard-Madoux M, Ober-Blobbaum JL, Costes L, et al. IL-10 control of CD11c+ myeloid cells is essential to maintain immune homeostasis in the small and large intestine. *Oncotarget.* 2016; 22: 32015-32030.
- Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol.* 2001; 19: 683-765.
- Nenna R, Tiberti C, Petrarca L, et al. The celiac iceberg: a characterization of the disease in primary school children. *J Pediatr Gastroenterol Nutr.* 2012; 56: 416-421.
- Nenna R, Guandalini S, Popp A, Kurppa K. Celiac disease. *Autoimmune Dis.* 2014; 2014: 623-784.
- Conceição-Machado ME, Santana ML, Silva RC, et al. Serologic screening of celiac disease in adolescents. *Rev Bras Epidemiol.* 2015; 18: 149-156.
- Setty M, Hormaza L, Guandalini S. Celiac disease. Risk assessment, diagnosis and monitoring. *Mol Diag Ther.* 2008; 12: 289-299.
- Agrawal S, Srivastava SK, Borkar M, Chaudhuri TK. Genetic affinities of north and northeastern populations of India: inference from HLA-based study. *Tissue Antigens.* 2008; 72: 120-130.
- Alaedini A, Green PH, Sander HW, et al. Ganglioside reactive antibodies in the neuropathy associated with celiac disease. *J Neuroimmun.* 2002; 127: 145-148.
- Aronsson C, Lee HS, Koletzko S, et al. TEDDY Study Group Effects of Gluten Intake on Risk of Celiac Disease: A Case-Control Study on a Swedish Birth Cohort. *Clin Gastroenterol Hepatol.* 2015; 14: 403-409.
- Bai D, Brar P, Holleran S, et al. Effect of gender on the manifestations of celiac disease: evidence for greater malabsorption in men. *Scand J Gastroenterol.* 2005; 40:183- 187.
- Meresse B, Malamut G, Cerf-Bensussan N. Celiac disease: an immunological jigsaw. *Immunity.* 2012; 36: 907-919.
- Cooke WT, Smith T. Neurological disorders associated with adult celiac disease. *Brain.* 1966; 89: 683-722.
- Giorgetti GM, Tursi A, Iani C. Assessment of autonomic function in untreated adult celiac disease. *World J Gastroenterol.* 2004; 10: 2715-2718.
- Briani C, Zara G, Alaedini A, et al. Neurological complications of celiac disease and autoimmune mechanisms: a prospective study. *J Neuroimmunol.* 2008; 195: 171-175.
- Barbato M, Curione M, Amato S, et al. Autonomic imbalance in celiac children. *Minerva Pediatr.* 2010; 62: 333-338.
- İşıkay S, Kocamaz H. The neurological face of celiac disease. *Arq Gastroenterol.* 2015; 52: 167-170.
- Hadjivassiliou M, Grunewald RA, Davies-Jones GA. Gluten sensitivity as a neurological illness. *J Neurol Neurosurg Psychiatry.* 2002; 71: 560-563.
- Gibbons CH, Freeman R. Autonomic neuropathy and coeliac disease. *J Neurol Neurosurg Psychiatry.* 2005; 76: 579- 581.
- Przybylska-Feluś M, Furgala A, Zwolińska-Wcisło M, et al. Disturbances of autonomic nervous system activity and diminished response to stress in patients with celiac disease. *J Physiol Pharmacol.* 2014; 65: 833-841.
- Volta U, De Giorgio R, Granito A, et al. Anti-ganglioside antibodies in coeliac disease with neurological disorders. *Dig Liver Dis.* 2006; 38: 183-187.
- Simons K, Ikonen E. Functional rafts in cell membrane. *Nature.* 1997; 387: 569-572.
- Díaz-Ramos A, Roig-Borrellas A, García-Melero A, López-Alemany R. α Enolase, a multifunctional protein: its role on pathophysiological situations. *J Biomed Biotechnol.* 2012; 2012: 156795.
- Godier A, Hunt BJ. Plasminogen receptors and their role in the pathogenesis of inflammatory, autoimmune and malignant disease. *J Thromb Haemost.* 2013; 11: 26-34.
- Lee KH, Chung HS, Kim HS, et al. Human α -enolase from endothelial cells as a target antigen of anti-endothelial cell antibody in Behcet's disease. *Arthritis Rheum.* 2003; 48: 2025-2035.
- Pratesi F, Moscato S, Sabatini A, et al. Autoantibodies specific for α enolase in systemic autoimmune disorders. *J Rheumatol.* 2000; 27: 109-115.
- Yang HB, Zheng WJ, Zhang X, Tang FL. Induction of endothelial cell apoptosis by anti- α -enolase antibody. *Chin Med Sci J.* 2011; 26: 152-157.
- Schuppan D, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. *Gastroenterology.* 2009; 137: 1912-1933.
- Setty M, Hormanza L, Guandalini S. Celiac disease. Risk assessment, diagnosis and monitoring. *Mol Diag Ther.* 2008; 12: 289-298.

- 32 Green PH, Murray JA. Routine duodenal biopsies to exclude celiac disease? *Gastrointestinal Endoscopy*. 2003; 58: 92-95.
- 33 Tursi A, Giorgetti GM, Iani C, et al. Peripheral neurological disturbances, autonomic dysfunction, and antineuronal antibodies in adult celiac disease before and after a gluten-free diet. *Dig Dis Sci*. 2006; 51: 1869-1874.
- 34 Latov N. Antibodies to glycoconjugates in neuropathy and motor neuron disease. *Prog Brain Res*. 1994; 101: 295-303.
- 35 Mizrahi A, Broide E, Buchs A, et al. Lack of correlation between disease activity and decreased stimulated secretion of IL-10 in lymphocytes from patients with celiac disease. *Scand J Gastroenterol*. 2002; 37: 924-930.
- 36 Núñez C, Alecsandru D, Varadé J, et al. Interleukin-10 haplotypes in Celiac Disease in the Spanish population. *BMC Med Genet*. 2006; 31: 32.
- 37 Salvati VM, Mazzarella G, Gianfrani C, et al. Recombinant human interleukin 10 suppresses gliadin dependent T cell activation in ex vivo cultured coeliac intestinal mucosa. *Gut*. 2005; 54: 46-53.
- 38 Cataldo F, Lio D, Marino V, et al. Plasma cytokine profiles in patients with celiac disease and selective IgA deficiency. *Pediatr Allergy Immunol*. 2003; 14: 320-324.
- 39 Leonard N, Hourihane DO, Whelan A. Neuroproliferation in the mucosa is a feature of coeliac disease and Crohn's disease. *Neurogastroenterol Motil*. 2008; 20: 884-890.
- 40 Papaioannou VE, Dragoumanis C, Theodorou V, et al. Relation of heart rate variability to serum levels of C-reactive protein, interleukin 6, and 10 in patients with sepsis and septic shock. *J Crit Care*. 2009; 24: 625-627.
- 41 Woiciechowsky C, Volk HD. Increased intracranial pressure induces a rapid systemic interleukin-10 release through activation of the sympathetic nervous system. *Acta Neurochir Suppl*. 2005; 95: 373-376.
- 42 Ghia JE, Blennerhassett P, Collins SM. Vagus nerve integrity and experimental colitis. *Am J Physiol Gastrointest Liver Physiol*. 2007; 293: 560-567.
- 43 Gottwald TP, Lhotak S, Stead RH. Effects of subdiaphragmatic vagotomy on mucosal mast cell densities in stomach and jejunum of rats. *Adv Exp Med Biol*. 1995; 371A: 303-306.
- 44 Rocco A, Sarnelli G, Compare D, et al. Tissue ghrelin level and gastric emptying rate in adult patients with celiac disease. *Dig Dis*. 2015; 33: 200-207.
- 45 Pinto-Sanchez MI, Bercik P, Verdu EF. Motility alterations in celiac disease and non-celiac gluten sensitivity. *Dig Dis*. 2015; 33: 200-207.

Stężenie przeciwciał przeciwko M1 gangliozydom, neuronoswoistej enolazy oraz interleukiny 10 jako potencjalnych markerów uszkodzenia autonomicznego układu nerwowego u chorych z celiakią

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SŁOWA KLUCZOWE

celiaki, interleukina 10, neuronoswoista enolaza, przeciwciała przeciwko M1 gangliozydom

STRESZCZENIE

WPROWADZENIE Celiakia jest enteropatią zapalną związaną z trwałą nietolerancją glutenu, charakteryzującą się objawami jelitowymi i pozajelitowymi, w tym objawami neurologicznymi. W przebiegu schorzeń neurologicznych oraz immunologicznych z manifestacją w układzie nerwowym wykazano obecność enzymu neuronoswoistej enolazy (NSE), interleukiny 10 (IL-10) oraz przeciwciał przeciwko M1 gangliozydom (GM1).

CELE Celem pracy była ocena stężeń IL-10, NSE oraz przeciwciał przeciwko GM1 w przebiegu celiakii oraz ich zależność względem zmian stwierdzonych w zapisie elektrogastrograficznym (EGG) oraz względem zmienności rytmu serca (*heart rate variability* – HRV).

PACJENCI I METODY Badaniem objęto 68 osób: 34 osoby z celiakią i 34 osoby zdrowe. Badano stężenie IL-10 i NSE oraz obecność przeciwciał przeciwko GM1 w surowicy. Oceniano korelacje między stężeniami IL-10, NSE i przeciwciał przeciwko GM1 a wynikami EGG oraz HRV.

WYNIKI W grupie pacjentów z celiakią obserwowano wyższy poziom przeciwciał przeciwko GM1 w porównaniu z grupą kontrolną (1,38 [0,98–2,03] ng/ml vs 0,81 [0,35–1,15] ng/ml). Mediana stężeń IL-10 w grupie pacjentów z celiakią różniła się istotnie od mediany w grupie kontrolnej (7 [4,33–11,48] pg/ml vs 4,27 [2,44–7] pg/ml; $p = 0,010$). W analizie HRV wykazano dodatnią korelację między stężeniem IL-10 a widmem bardzo niskiej częstotliwości ($r = 0,63$; $p = 0,003$). Nie wykazano zależności między stężeniami IL-10, NSE i przeciwciał przeciwko GM1 a parametrami EGG.

WNIOSKI Przewlekły stan zapalny w przebiegu CD może być przyczyną zmian w obrębie autonomicznego układu nerwowego oraz rozwoju zaburzeń neurologicznych. Przeciwciała przeciwko GM1 oraz IL-10 mogą być więc wskaźnikami uszkodzenia układu nerwowego w przebiegu celiakii

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