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ORIGINAL ARTICLE

The Performance of Nesfatin-1 in Distinguishing Irritable Bowel Syndrome Presenting Predominantly with Diarrhea from Celiac Disease

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SUMMARY

Background: **We hypothesized that nesfatin-1, an anti-inflammatory peptide, could be used as a non-invasive diagnostic tool in the identification of celiac disease (CD) and irritable bowel syndrome presenting predominantly with diarrhea (IBS-D).**
Methods: **Thirty-five patients with IBS-D who met the Rome III criteria, 28 patients with celiac disease who met the diagnostic criteria of the Marsh-Oberhuber classification, and 30 age- and gender-matched healthy controls were included in this cross-sectional study. All subjects responded to the IBS Severity Scoring System (IBS-SSS) questionnaire that was used to determine pain severity, pain frequency, bloating, dissatisfaction with bowel habits, and life interference.**
Results: **Nesfatin-1 levels were significantly higher in the CD group compared to the IBS-D group and healthy controls. Nesfatin-1 was also higher in the IBS-D group compared to controls. Nesfatin-1 levels were correlated with IBS-SSS ($r = 0.884$, $p < 0.001$), severity of abdominal pain and discomfort ($r = 0.644$, $p < 0.001$), and C-reactive protein concentrations ($r = 0.303$, $p = 0.004$). ROC curve analysis demonstrated that a cutoff value of > 98.1 pg/mL for nesfatin-1 could discriminate subjects with CD from those with IBS-D and also healthy controls with a sensitivity of 82% and a specificity of 80%.**
Conclusions: **The results of this study show that subjects with CD have higher nesfatin-1 levels compared to those with IBS-D or to the healthy controls. Moreover, nesfatin-1 can discriminate subjects with CD from those with IBS-D and also healthy controls, with high sensitivity and specificity. Further studies with histopathological evaluation are required to clearly address the role of nesfatin-1 in the diagnosis of CD.**
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KEY WORDS

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INTRODUCTION

Irritable bowel syndrome (IBS) is a disorder characterized by abdominal pain or discomfort associated with altered bowel habits in the absence of an organic cause. Population-based studies have shown that IBS affects up to 10 - 15% of the general population [1]. Irritable bowel syndrome presenting predominantly with diar-

rhea (IBS-D) is a clearly-defined subtype of IBS which has been reported to significantly impair health-related quality of life, work productivity, and the activity of affected individuals [2].

Celiac disease (CD), which is accepted to be associated with the effects of environmental factors in genetically susceptible individuals, is an autoimmune disorder causing duodenal villous atrophy leading to abdominal symptoms ranging from severe malabsorption to minimally symptomatic disease [3]. Various antibodies have been shown to be responsible for the majority of the intestinal and extraintestinal symptoms [4]. Chronic diarrhea, weight loss, chronic fatigue, abdominal pain, and growth disturbances are the typical symptoms of CD. The diagnosis of CD in adults is based on serology results and duodenal biopsy sampling - which involves an invasive procedure [5].

Currently, there is no definitive diagnostic test for IBS, and the diagnosis is frequently based on medical history, physical examination, and rule-out tests targeting IBS-like disorders, including CD, non-celiac gluten sensitivity, lactose intolerance, small intestinal bacterial overgrowth, α-amylase/trypsin inhibitors, and allergic contact mucositis [6]. However, the diarrhea subtype of IBS can be further confused with celiac disease (CD) due to the overlapping symptoms such as chronic diarrhea, bloating, and abdominal pain, which are mostly food-dependent in both diseases. It has been reported that nearly 10% of CD patients are misdiagnosed as IBS prior to receiving proper diagnosis [7]. An accurate diagnosis is therefore crucial to implement disease-specific therapeutic options. As such, non-invasive or minimally-invasive diagnostic tools that can facilitate discrimination of IBS-D from CD may be critical to avoid intestinal biopsy and its complications.

Nesfatin-1, an 82 amino acid polypeptide, was first described as having a role in the appetite-control hypothalamic nuclei of rats [8]. Further studies demonstrated the presence of Nesfatin-1 in other tissues, including gastric mucosa. NUCB2 mRNA expression (causing nesfatin-1 production) has been shown to be increased in the stomach, compared to the brain or other peripheral organs [9]. Recent data indicate that nesfatin-1 production and release might be associated with inflammatory status and peripheral inflammatory signals [10]. Moreover, nesfatin-1 has been shown to alleviate indomethacin-induced gastric injury, likely through its anti-inflammatory properties [11].

Given the role of inflammation in the development of symptoms in both IBS-D and CD, we hypothesized that nesfatin-1, with its anti-inflammatory properties, could be used as a non-invasive diagnostic tool in the identification of IBS-D and CD. The present study aimed to investigate nesfatin-1 levels in IBS-D and CD and to evaluate whether nesfatin-1 levels could be used in the differentiation of these two clinically similar diseases.

MATERIALS AND METHODS

The present cross-sectional, single-center study was conducted on patients with IBS or CD who were followed at the Gaziosmanpaşa Taksim Educational and Research Hospital, Department of Gastroenterology. All consecutive subjects, either with IBS-D or CD, were enrolled in the study if they were aged ≥ 18 years. Thirty-five patients with IBS-D who met the Rome III criteria, 28 patients with CD who met the diagnostic criteria of the Marsh-Oberhuber classification, and 30 age- and gender-matched healthy controls were included in this cross-sectional study [12,13]. Patients that were pregnant, those who had heart failure, malignancy, advanced liver or kidney disease, hepatitis B and C infection, and those younger than 18 years were excluded. Written informed consent was obtained from all participants included in the study. The study was approved by the Institutional Ethical Committee and was performed in accordance with the most recent version of the Helsinki Declaration (148/16.10.2019).

Age, gender, and body mass index (BMI) were recorded for all patients with IBS-D and CD. Blood samples, following 12 hours of fasting, were drawn from all participants for complete blood count, C-reactive protein, and nesfatin-1 measurements. After centrifugation (4,000 RPM for 10 minutes), 1 to 2 mL serum samples were stored at -80°C until assayed. An enzyme-linked immunosorbent assay (ELISA) kit was used to measure the serum levels of nesfatin-1 (Boster Biological Technology Corporation, Wuhan, China). The rest of the parameters were measured with routine devices by the biochemistry laboratory.

IBS symptom score

All subjects were requested to respond to the IBS Severity Scoring System (IBS-SSS) questionnaire to evaluate five dimensions over the prior 10 days: pain severity, pain frequency, bloating, dissatisfaction with bowel habits, and life interference [14]. Subjects answered each question on a 100-point visual analogue scale (VAS). Since the test consists of 5 questions, IBS-SSS ranges were between 0 and 500. Scores of 75 - 175, 175 - 300, and > 300 were defined as mild, moderate, and severe, respectively. Patients with IBS who reported a score below 75 were considered to be in remission.

Primary outcome

The difference in nesfatin-1 levels between subjects with IBS-D, CD, and healthy controls was the primary outcome measure of this study. The association of nesfatin-1 level and symptom severity was the secondary outcome measure.

Statistical analysis

All analyses were performed on SPSS v 21 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used for determining whether variables were normally distributed. Homogeneity of variances was assessed with

Table 1. Summary of individuals' characteristics according to groups.

	IBS-D (n = 33)	Healthy Controls (n = 30)	Celiac Disease (n = 28)	p
Age	34.79 ± 9.37	36.23 ± 9.48	33.39 ± 7.53	0.479 *
Gender				
Female	12 (36.36%)	17 (56.67%)	14 (50.00%)	0.256 &
Male	21 (63.64%)	13 (43.33%)	14 (50.00%)	
Height	165.36 ± 9.92	162.97 ± 10.04	164.93 ± 8.15	0.574 *
Weight	65.45 ± 9.94 [a]	62.32 ± 10.56 [a]	53.66 ± 6.98 [b]	< 0.001 *
Body Mass Index	23.94 ± 3.11 [a]	23.53 ± 3.91 [a]	19.68 ± 1.52 [b]	< 0.001 *
Abdominal Pain and Discomfort				
Absent	0 (0.00%) [a]	24 (80.00%) [b]	0 (0.00%) [c]	< 0.001 &
Mild	6 (18.18%)	5 (16.67%)	0 (0.00%)	
Relevant	10 (30.30%)	1 (3.33%)	7 (25.00%)	
Severe	11 (33.33%)	0 (0.00%)	17 (60.71%)	
Extremely Severe	6 (18.18%)	0 (0.00%)	4 (14.29%)	
Bowel Movements (week)	25 (18 - 32) [a]	7 (7 - 9) [b]	28 (18 - 46) [a]	< 0.001 ‡
White Blood Cell (x 1,000)	6.90 ± 1.61 [a]	6.46 ± 1.26 [ab]	5.66 ± 2.17 [b]	0.021 *
Hemoglobin	14.17 ± 1.75 [a]	13.87 ± 1.67 [a]	10.07 ± 0.88 [b]	< 0.001 *
Platelet (x 1,000)	254 (129 - 486)	267 (165 - 570)	244.5 (131 - 459)	0.190 ‡
CRP	5.47 (2.10 - 8.65) [a]	2.51 (0.16 - 32.97) [b]	4.60 (1.80 - 10.46) [a]	< 0.001 ‡
Nesfatin	89.26 (39.48 - 189.69) [a]	4.49 (1.43 - 17.12) [b]	140.83 (72.33 -851.98) [c]	< 0.001 ‡
IBS-SSS	220 (100 - 360) [a]	62.5 (35 - 80) [b]	322.5 (170 - 486) [a]	< 0.001 ‡
IBS-SSS				
Absent	0 (0.00%) [a]	30 (100%) [b]	0 (0.00%) [c]	< 0.001 &
Mild	8 (24.24%)	0 (0.00%)	2 (7.14%)	
Moderate	14 (42.42%)	0 (0.00%)	8 (28.57%)	
Severe	11 (33.33%)	0 (0.00%)	18 (64.29%)	

Data are given as mean ± standard deviation or median (minimum-maximum) for continuous variables according to normality and fraquency (percentage) for categorical variables.
Same letters denote lack of significant difference between groups.
IBS-SSS - The Severity Scoring System in IBS, CRP - C reactive protein.
*** - ANOVA test, & - Chi-square test, ‡ - Kruskal-Wallis test.**

the Levene test. Data are given as mean ± standard deviation or median (minimum-maximum) for continuous variables with regard to normality, and frequency (percentage) for categorical variables. Normally distributed variables (age, height, weight, BMI, white blood cell, and hemoglobin) were compared with regard to disease groups through the one-way analysis of variances (ANOVA) test. Non-normally distributed variables (frequency of bowel movements, platelet count, CRP, nesfatin-1 and IBS-SSS) were compared with the Kruskal-Wallis test. The Tukey test or Tamhane test or Bonferroni correction method were used for pairwise comparisons depending on normality of distribution and the variances homogeneity of the variables. Categorical variables were analyzed with the Chi-square test. Spearman's correlation coefficients were calculated for the assessment of the relationships between variables. $p < 0.05$ was defined as the level of statistical significance.

RESULTS

Thirty subjects with IBS-D (mean age 34.79 ± 9.37 years, 36.36% male), 28 subjects with CD (mean age 33.39 ± 7.53 years, 50% male), and 30 healthy controls (mean age 36.23 ± 9.48 years, 56.67% male) were enrolled in the study. The demographic and clinical features of the study groups are presented in Table 1. The groups were similar with respect to age, gender and platelet count. BMI was significantly higher in the CD

Table 2. Relationship between variables.

		Nesfatin	IBS-SSS
Nesfatin	**r**		**0.884**
	p		**< 0.001**
Age	**r**	**-0.041**	**-0.071**
	p	**0.699**	**0.503**
Height	**r**	**-0.013**	**0.066**
	p	**0.901**	**0.533**
Weight	**r**	**-0.286**	**-0.189**
	p	**0.006**	**0.073**
Body Mass Index	**r**	**-0.302**	**-0.259**
	p	**0.004**	**0.013**
Severity of Abdominal Pain Discomfort	**r**	**0.644**	**0.685**
	p	**< 0.001**	**< 0.001**
Frequency of Bowel Movements (week)	**r**	**0.739**	**0.725**
	p	**< 0.001**	**< 0.001**
White Blood Cell	**r**	**-0.153**	**-0.142**
	p	**0.147**	**0.178**
Hemoglobin	**r**	**-0.462**	**-0.458**
	p	**< 0.001**	**< 0.001**
Platelet	**r**	**-0.137**	**-0.066**
	p	**0.196**	**0.532**
CRP	**r**	**0.303**	**0.259**
	p	**0.004**	**0.013**

r - Spearman's Correlation Coefficient.
IBS-SSS - The IBS Severity Scoring System, CRP - C reactive protein.

group compared to the IBS-D group and healthy controls. Severe abdominal pain was more frequent in subjects with CD, compared to subjects with IBS-D and the healthy controls. Leukocyte count was significantly lower in CD subjects compared to healthy controls. Hemoglobin level was also significantly lower in subjects with CD compared to those with IBS-D and to the healthy controls. C-reactive protein was similar in subjects with CD and IBS-D. Moreover, IBS-SSS scores, as a quantitative measure of the symptom severity, were similar in subjects with CD and IBS-D. However, the nesfatin-1 level was significantly higher in the CD group compared to the IBS-D group and the healthy controls. The nesfatin-1 levels of the IBS-D group were also higher than that of the healthy controls.

The correlations between selected variables (according to significance and correlation coefficients) and the values of IBS-SSS and nesfatin-1 are given in Table 2. IBS-SSS was significantly correlated with nesfatin-1 level ($r = 0.884$, $p < 0.001$) and C-reactive protein ($r = 0.259$, $p = 0.013$) levels, while it was negatively correlated with hemoglobin level ($r = -0.458$, $p < 0.001$) and BMI ($r = -0.259$, $p = 0.013$). Nesfatin-1 level was significantly correlated with the frequency of bowel movements ($r = 0.739$, $p < 0.001$), severity of abdominal pain and discomfort ($r = 0.644$, $p < 0.001$), and C-reactive protein concentration ($r = 0.303$, $p = 0.004$). Nesfatin-1 level was negatively correlated with BMI ($r = -0.302$, $p = 0.004$) and hemoglobin level ($r = -0.462$, $p < 0.001$). ROC curve analysis demonstrated that a cutoff value of > 98.1 pg/mL for nesfatin-1 could discriminate subjects with CD from those with IBS-D and healthy controls with a sensitivity of 82% and specificity of 80% (AUC: 0.899, 95% CI: 0.837 - 0.962, $p < 0.001$) (Figure 1).

DISCUSSION

Celiac disease and IBS-D are two disorders sharing similar clinical presentations. We aimed to investigate whether nesfatin-1, an appetite-controlling peptide with anti-inflammatory properties, could be utilized for the discrimination of these two disorders. The present study shows that subjects with CD have higher nesfatin-1 levels compared to those with IBS-D or to the healthy controls. The nesfatin-1 level is positively correlated with symptom severity and C-reactive protein level and was negatively correlated with BMI and hemoglobin levels. Moreover, our findings indicate that nesfatin-1 can discriminate subjects with CD from those with IBS-D (and also healthy controls) with high sensitivity and specificity.

Celiac disease and IBS-D are often indistinguishable as a consequence of the overlapping clinical features, including chronic diarrhea, bloating, and abdominal pain. Fluid-dependent symptom development also complicates the discrimination of the two disorders. Celiac disease is, therefore, acknowledged as an IBS-like disorder which needs to be identified correctly in order to be able to implement specific treatment options [15]. However, currently, definite diagnosis of CD is based on the demonstration of villous atrophy via intestinal biopsy - a highly invasive procedure. Several serological markers, including antibodies against gliadin, endomysium, tissue transglutaminase, and deamidated gliadin peptide, have been introduced for the screening of CD [16]; however, even if serological testing is positive, the diagnosis of CD still needs to be confirmed by endoscopic small intestinal biopsy [17]. Moreover, the diagnostic accuracy of these tests may vary and are often insufficient to accurately detect subjects with CD. In a recent meta-analysis of 26 studies that enrolled subjects with biopsy-confirmed CD, the sensitivity of antibodies against endomysium and tissue transglutaminase for detecting persistent villous atrophy was reported to be below 50% [18]. Furthermore, serological tests may also be influenced by age, elimination of gluten from the diet, selective immunoglobulin A deficiency, and use of corticosteroids or immunomodulatory drugs [19]. When the chronic nature and substantial lifelong implications

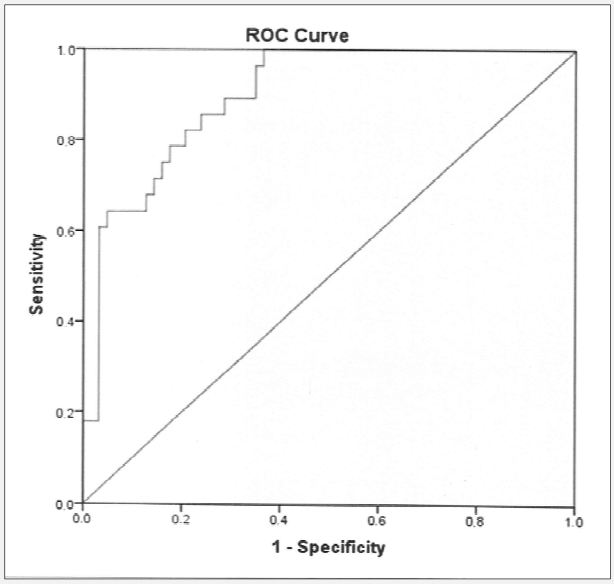

Figure 1. ROC curve demonstrating the performance of nesfatin-1 in identification of subjects with celiac disease.

are taken into account, it appears that there is still a need for the identification of further non-invasive tests to facilitate the diagnosis of CD.

Nesfatin-1, an 82-amino-acid polypeptide, was initially detected in several brain areas of rats, including the arcuate nucleus, supraoptic nucleus, hypothalamic area and the brain stem, and was shown to have a role in the integration of feeding and metabolic functions [20]. Further studies have demonstrated that amino-terminal fragment mRNA expression for nesfatin-1 was much higher in the rat stomach than the brain [9]. Moreover, some previous data suggested an association between peripheral nesfatin-1 release and the presence of inflammation. An interesting study conducted by Ozturk et al. has revealed that administration of nesfatin-1 promotes healing in rats with acetic acid-induced colitis via its anti-inflammatory and antioxidant properties, which apparently occur via oxytocin and ghrelin receptors [21]. Kolgazi et al. reported that in rats with acetic-acid induced gastric ulcer, the treatment with nesfatin-1 decreases neutrophil migration and the levels of inflammatory mediators, most probably via a cyclooxygenase-dependent mechanism [22]. The study of Szlachcic and colleagues suggests that nesfatin-1 displays its healing effects in chronic gastric ulcers through the improvement in gastric blood flow and mucosal restoration [23]. Recent data indicate that nesfatin-1 production and release might be related with inflammatory status and peripheral inflammatory signals [24]. Tatar et al. suggested a positive correlation between nesfatin-1 values and pathology score in their ischemic colitis model. However, the underlying mechanism is not clearly explained [25]. Kvlividze et al. have shown that patients with rheumatoid arthritis that serum nesfatin-1 concentrations were correlated with a high level of C-reactive protein and erythrocyte sedimentation rate [26].

In light of the limited data available, clinical or experimental evidence demonstrating the action of nesfatin-1 in CD or IBS-D is lacking. To the best of our knowledge, the present study is the first to demonstrate the performance of nesfatin-1 in the diagnosis of CD and its possible role in distinguishing between CD and IBS-D. Our findings show that nesfatin-1 not only discriminates subjects with CD from those with IBS-D and healthy controls with high sensitivity and specificity, but also correlates with symptom severity. Although the mechanism underlying the increase in nesfatin-1 in subjects with CD is beyond the scope of this study, we suggest that the underlying inflammation in subjects with CD might be the primary cause of increased nesfatin-1. The correlation between the nesfatin-1 and C-reactive protein as a measure of inflammation, supports this suggestion. The study has some limitations to be mentioned. The sample size is relatively small to come to a clear conclusion regarding the role of the nesfatin-1 in the identification of subjects with CD and in discrimination of subjects with CD from those with IBS-D. The lack of histological data and measurement of pro-inflammatory markers prevents reaching a clear conclusion regarding the underlying mechanism of nesfatin-1 increase in pa-

tients with CD. Further studies with larger sample size and histopathological evaluation are required to address the role of nesfatin-1 in the diagnosis of CD.

CONCLUSION

Nesfatin-1 can be used in the identification of subjects with CD and in discrimination of subjects with CD from those with IBS-D. The correlation between nesfatin-1 and C-reactive protein levels suggests inflammation to be the primary cause of increased nesfatin-1. Future studies could benefit from performing tissue-based comparisons of nesfatin-1 expression in patients with CD and IBS-D.

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Ethical Approval:
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent:
Informed consent was obtained from all individual participants included in the study.

Author Contributions:
Eylem Karatay performed collected data. Özlem Gül Utku performed data collection and wrote the article. Neval Aksoy performed the biochemical study.

Declaration of Interest:
The authors declare that they have no conflict of interest.

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