Relationship between irritable bowel syndrome and plasma and tissue ghrelin levels

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ABSTRACT

Background/Aims: This study aimed to evaluate the relationship between irritable bowel syndrome (IBS) and plasma and tissue ghrelin levels.

Materials and Methods: Patients who had undergone gastroscopy procedure for any reason previously were enrolled in the study. Among these, patients with IBS symptoms were evaluated according to the Roma III criteria. The healthy control group comprised patients with no IBS symptom and had undergone gastroscopy procedure for another reason. The plasma ghrelin level and tissue ghrelin level obtained by immunohistochemical examination of biopsy specimens taken from the gastric antrum and corpus were evaluated in all participants.

Results: The mean age of 90 participants was 43.64±12.64 years. The median value of the plasma ghrelin level was 3.29 (1.2-12.7) in the diarrhea group (IBS-D), 1.49 (0.82-7.08) in the constipation group (IBS-C), and 1.5 (0.2-3.7) in the control group. The plasma ghrelin levels between the groups were found to be significantly higher in IBS-D than in IBS-C and the control groups (p=0.001 and p=0.001, respectively). On comparing antral mucosal gland biopsy outcomes among the groups, staining intensity score was found to be significantly high in IBS-C as compared with the control group, whereas no significant difference was observed between IBS-D and the control groups (p=0.020 and p=0.429, respectively).

Conclusion: The plasma ghrelin level in IBS-D and the staining intensity in the antral mucosal gland in IBS-C were found to be significantly higher. In addition, there was no difference between the groups in terms of ghrelin staining intensity in the gastric corpus.

Keywords: Irritable bowel syndrome, ghrelin, motilin related peptide

INTRODUCTION

Irritable bowel syndrome (IBS) is a disease characterized by abdominal pain, distension and gas with psychosocial factors, impaired motility, past infections, genetic factors, impaired intestinal flora, mucosal inflammation, and gastrointestinal system hormones playing a role in the etiopathogenesis (1-3). The worldwide prevalence of IBS varies from 0.8% to 31.6%; similarly, it ranges between 6.3% and 10.2% in Turkey (4). IBS imposes an important economic burden on the society (5). The annual total cost of IBS is approximately $30 billion, and it is more expensive than that of other common chronic diseases (5). Ghrelin, one of the hormones playing a role in the pathogenesis of IBS, is a 28-amino acid lipopeptide hormone produced in the enteroendocrine X/A-like cells of the gastric oxyntic gland mucosa (6). Ghrelin is found in many tissues and organs in the body, such as stomach, pancreas, kidney, liver, hypothalamus and pituitary glands, and in immune cells (7). There are studies demonstrating the direct effect of ghrelin hormone, which is the highest in the stomach, on gastrointestinal system secretions and motility (6,8,9). The present study aimed to determine the relationship between IBS and the plasma/tissue ghrelin level.

MATERIALS AND METHODS

Study universe

Participants aged between 18 and 65 years who visited Dr. Lütfi Kırdar Training and Research Hospital Gastroenterology Policlinics between May 2014 and 2015 and had previously undergone gastroscopy procedure for any rea-

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son were enrolled in the study. Participants with IBS symptoms were evaluated according to the Roma III criteria. The first 90 patients with IBS symptoms comprised the study group and the rest of the patients without IBS symptoms comprised the control group. The study group was further subdivided into two groups as: diarrhea predominant IBS (IBS-D) and constipation predominant IBS (IBS-C). Gastroscopic biopsy samples of all participants were immunohistochemically stained and examined for ghrelin cell intensity. Participants who underwent gastroscopy procedure at our polyclinic during the same time and had no IBS symptoms were classified into the control group.

Measurements

The plasma ghrelin levels of the participants were measured by the ELISA method (Ghrelin ELISA kit, Tecan Sunrise 093002343, Hanzgzhou Eastbiopharm) in the venous blood samples obtained in the morning after a 12-h fasting period. The tissue ghrelin level was assessed immunohistochemically via ghrelin antibody (1:300 dilution, Abcam Inc., UK) expression in the paraffin-embedded gastric biopsy specimens taken from the gastric antrum and corpus (curvature minor and major) of all participants. All the gastric biopsies were evaluated by a single pathologist blinded to the clinical data. In all biopsy materials prepared by the immunohistochemical staining method (BondMax, Leica, Wetzlar, Germany), six separate points were given to the staining intensity at the surface epithelium, mucosal glands, and stroma of both the gastric antrum and gastric corpus. During scoring, extensive staining was considered as 3 points, less intensive staining at more than one focus was considered as 2 points, staining in a few cells in one focus was considered as 1 point, and no staining was considered as 0 points.

Exclusion criteria

Participants with mixed-type IBS and persons with a history of previous gastrointestinal surgery documented malignancy, IBD, diabetes mellitus, atrophic gastritis, celiac disease, chronic liver disease, or chronic kidney disease, as well as pregnant women were excluded from the study.

Statistical analysis

Statistical analyses of data was performed using the SPSS 22 program (IBM Corp.; Armonk, NY, USA). Parameters for normal distribution were evaluated by the Shapiro-Wilks test. Descriptive statistics were evaluated as frequency, percentage, mean±standard deviation, and median. One-way ANOVA test and Student t-test were performed for normally distributed continuous variables, and the results were presented as frequency, mean±standard deviation, percentage, and median. Furthermore, Mann-Whitney U test, Kruskal-Wallis test, and Spearman correlation test were used for the comparison of continuous variables with abnormal distribution. In addition, chi-square test and Fisher exact test were used for the analysis of categorical

Table 1. Sociodemographic characteristics of the constipation IBS, diarrhea IBS, and control groups

	IBS-C Mean±SD (n=30)	IBS-D Mean±SD (n=30)	Control Mean±SD (n=30)	p[a]
Age	42.47±10.40	39.87±11.05	48.60±14.79	0.021
BMI	28.93±4.02	27.41±3.87	26.99±3.35	0.117
	n (%)	n (%)	n (%)	p[b]
Gender				
Female	25 (83.3%)	10 (33.3%)	17 (56.7%)	0.001
Male	5 (16.7%)	20 (66.7%)	13 (43.3%)	
Education				
Primary School or Lower	21 (70.0%)	16 (53.3%)	16 (53.3%)	0.755
Middle-School	3 (10.0%)	7 (23.3%)	5 (16.7%)	
High School	4 (13.3%)	5 (16.7%)	7 (23.3%)	
University	2 (6.7%)	2 (6.7%)	2 (6.7%)	
Smoking	6 (20.0%)	8 (26.7%)	10 (33.3%)	0.506
Alcohol	0 (0.0%)	1 (3.3%)	1 (3.3%)	1.000

BMI: body mass index; SD: standard deviation; IBS-C: constipation predominantly irritable bowel syndrome; IBS-D: diarrhea predominantly irritable bowel syndrome
[a]One way ANOVA Test, [b]Chi-square test and Fisher Exact test

Table 2. Distribution of immunohistochemical staining scores of the corpus biopsy specimens among the groups

Corpus		IBS-C (n=30) n (%)	IBS-D (n=30) n (%)	Control (n=30) n (%)	p[a]
Surface Epithelium	Minimal Staining	1 (100.0%)	3 (100.0%)	5 (100.0%)	-
	Moderate Staining	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Extensive Staining	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Staining Intensity Score	1 (3.3%)	3 (10.0%)	5 (16.7%)	0.284
Mucosal gland	Minimal Staining	12 (52.2%)	11 (45.8%)	8 (34.8%)	0.672
	Moderate Staining	10 (43.5%)	12 (50.0%)	12 (52.2%)	
	Extensive Staining	1 (4.3%)	1 (4.2%)	3 (13.0%)	
	Staining Intensity Score	23 (76.7%)	24 (80.0%)	23 (76.7%)	1.000
Stroma	Minimal Staining	1 (100.0%)	6 (100.0%)	3 (100.0%)	-
	Moderate Staining	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Extensive Staining	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Staining Intensity Score	1 (3.3%)	6 (20.0%)	3 (10.0%)	0.146

IBS-C: constipation predominantly irritable bowel sydrome; IBS-D: diarrhea predominantly irritable bowel sydrome
[a]Fisher Exact test

Table 3. Distribution of ghrelin immunohistochemical staining scores of the antrum biopsy specimens among the groups

Antrum		IBS-C (n=30) n (%)	IBS-D (n=30) n (%)	Control (n=30) n (%)	p[a]
Surface Epithelium	Minimal Staining	1 (100.0%)	3 (100.0%)	5 (100.0%)	0.284
	Moderate Staining	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Extensive Staining	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Staining Intensity Score	1 (3.3%)	3 (10.0%)	5 (16.7%)	-
Mucosal gland	Minimal Staining	18 (90.0%)	12 (75.0%)	10 (100.0%)	0.199
	Moderate Staining	2 (10.0%)	4 (25.0%)	0 (0.0%)	
	Extensive Staining	0 (%0.0)	0 (0.0%)	0 (0.0%)	
	Staining Intensity Score	20 (66.7%)	16 (53.3%)	10 (33.3%)	0.038
Stroma	Minimal Staining	2 (100.0%)	3 (75.0%)	5 (100.0%)	0.540
	Moderate Staining	0 (0.0%)	1 (25.0%)	0 (0.0%)	
	Extensive Staining	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Staining Intensity Score	2 (6.7%)	4 (13.3%)	6 (20.0%)	0.374

IBS-C: constipation predominantly irritable bowel sydrome; IBS-D: diarrhea predominantly irritable bowel sydrome
[a]Fisher Exact test

variables. A p value of <0.05 was considered as statistically significant. The study was approved by the Ethics Committee of Dr. Lütfi Kırdar Training and Research Hospital, and informed consents were obtained from all participants.

RESULTS

In total, 90 participants with a mean age of 43.64±12.64 years and 52 (57.8%) were female were included in the study. The distribution of the sociodemographic characteristics of the participants among IBS-D, IBS-C, and the control groups is summarized in Table 1.

The mean age was 41.19±10.63 years in the IBS group and 48.60±14.79 years in the control group (p=0.007). The mean age was found to be significantly lower in IBS-D than in the control group, but no significant differ-

ence was determined between IBS-D and IBS-C or control group (p=0.021, p=0.690, and p=0.133, respectively). Moreover, female participants were significantly higher in number in IBS-C than in the other groups, whereas there was no difference between IBS-D and the control group (p=0.001 and p=0.119).

No significant relationship was determined between the plasma ghrelin level and age or gender of the participants (p=0.701 and p=0.738, respectively). In addition, there was no significant relationship between the plasma ghrelin level and smoking or alcohol consumption (p=0.809 and p=0.060, respectively). The median ghrelin level was 3.29 (1.2-12.7) in IBS-D, 1.49 (0.82-7.08) in IBS-C, and 1.5 (0.2-3.7) in the control group. The plasma ghrelin level was found to be significantly higher in IBS-D than in IBS-C and the control group (p=0.001 and p=0.001); however, a similar difference was not found between IBS-C and the control group (p=0.156).

Corpus and antral mucosal gland biopsies were compared between the groups, and the staining intensity score was found to be significantly higher in IBS-C than in the control group (p=0.020), but no significant difference was determined between IBS-D and IBS-C or the control group (p=0.429 and p=0.193, respectively). The comparison of the groups in terms of immunohistochemical staining scores of ghrelin cells in the gastric corpus and antrum biopsy specimens is summarized in Tables 2 and 3.

DISCUSSION

Irritable bowel syndrome is a functional bowel disease characterized by abdominal discomfort, distension, and pain, but the pathophysiology of symptoms remains unclear (1,6,10). Numerous gastrointestinal peptides, such as serotonin, gastrin, obestatin, cholecystokinin, and motilin, take place in the intestinal functions and gastric emptying (6). Nevertheless, recent studies indicate that ghrelin hormone influences gastrointestinal motility (6,9). The present study evaluated the relationship between IBS and plasma and tissue ghrelin hormone levels.

Ghrelin is a hormone secreted mainly from the stomach and involves many different systems including nutrition, gastric acid secretion, gastric motility, and cell proliferation (6). In a study, the plasma ghrelin level was found to be significantly higher in females, whereas another study determined no significant difference between the genders in terms of the plasma ghrelin levels (11,12). The present study determined no significant relationship between the plasma ghrelin level and gender.

Although some studies determined a negative correlation between the age and ghrelin level, some studies failed to determine such a relationship (12,13). The present study found no relationship between the age and plasma ghrelin level. In addition, the mean age of IBS-D was statistically significantly lower than that of the control group. There was no significant difference between IBS-C and other groups. This suggests that the plasma ghrelin level, which was higher in IBS-D than in the other two groups (IBS-C and control), alone is not associated with lower age.

In a study, no significant relationship was determined between the plasma ghrelin level and alcohol consumption (14). Likewise, the present study failed to determine a significant relationship between the plasma ghrelin level and smoking or alcohol consumption in either group.

A study found a significantly higher plasma ghrelin level in patients with celiac disease than in patients in IBS-D and healthy control group, whereas a similar relationship could not be observed between patients in IBS-D and those in the healthy control group (15). Although another study found no significant difference between patients with IBS and those in the healthy control group in terms of total plasma ghrelin level, the octanyl ghrelin level was found to be higher in the IBS group (16). In the present study, the plasma ghrelin level was significantly higher in IBS-D, but there was no difference between other groups.

Ghrelin has been shown to increase gastrointestinal motility (9,15-19). Tebbe et al. (19) reported that ghrelin microinjected into the paraventricular nucleus increased propulsive colonic motor activity, as indicated by shortened colonic transit time in rats, and this suggests that the effect of ghrelin on colonic motility is mediated via central hypothalamic mechanisms. A study determined a significantly higher ghrelin intensity in the gastric mucosal biopsy specimens of IBS-D versus IBS-C and control groups (17). In the same study, no significant difference was determined between the groups in terms of the plasma ghrelin level (17). Another study evaluated gastric corpus biopsy specimens between patients with IBS and those in the healthy control group and found the ghrelin intensity to be significantly different in IBS-D versus IBS-C and the control group (18). Moreover, the same study observed a positive correlation between the severity of diarrhea and ghrelin cell intensity (18). In the present study, antrum biopsy results revealed a significantly higher ghrelin staining intensity in the mucosal gland of the constipation group than in the control group, whereas a similar difference was not observed in terms of corpus biopsy.

A limitation of the study was that ghrelin hormone has different subgroups, and all subgroups were not measured in the study. In addition, the use of semi-quantitative scoring is another limitation of this study.

In conclusion, although there are studies demonstrating the effects of ghrelin hormone on gastrointestinal system motility, the relation of ghrelin hormone with IBS has not been clarified yet (6,15,18). This study shows that the plasma ghrelin level was higher in IBS-D, whereas the ghrelin level in the antral mucosal gland was higher in IBS-C. On the other hand, there was no difference between the groups in terms of staining intensity in the corpus region. These results suggest that there is a link between the IBS subgroups and ghrelin levels in the plasma and mucosal gland. Ghrelin level differences in the IBS subgroups may be due to the effect of the structure of ghrelin or level of receptor.

Ethics Committee Approval: Ethics Committee Approval has received for this study from the Ethics Committee of Dr. Lütfi Kırdar Research and Training Hospital.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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References

1. Agreus L, Svardsudd K, Nyren O, Tibblin G. Irritable bowel syndrome and Dyspepsia in the general population: overlap and lack of stability over time. Gastroenterology 1995; 109: 671-80. [CrossRef]

2. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology 2006; 130: 1480-91. [CrossRef]

3. El-Salhy M, Gundersen D, Gilja OH, Hatlebakk JG, Hausken T. Is irritable bowel syndrome an organic disorder? World J Gastroenterol 2014: 14; 20: 384-400. [CrossRef]

4. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. Clinical Epidemiology 2014: 6: 71-80.

5. Hulisz D. The Burden of Illness of Irritable Bowel Syndrome: Current Challenges and Hope for the Future. J Manag Care Pharm 2004; 10: 299-309. [CrossRef]

6. Cheung CK, Wu JC. Role of ghrelin in the pathophysiology of gastrointestinal disease. Gut Liver 2013; 7: 505-12. [CrossRef]

7. Wang G, Lee HM, Englander E, Greeley GH Jr. Ghrelin-not just another stomach hormone. Regul Pept 2002; 105: 75-81. [CrossRef]

8. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev 2005; 85: 495-522. [CrossRef]

9. Müller TD, Nogueiras R, Andermann ML, et al. Ghrelin. Molecular Metabolism 2015; 4: 437-60. [CrossRef]

10. Barbara G, De Giorgio R, Stanghellini V, Cremon C, Salvioli B, Corinaldesi R. New Pathophysiological mechanisms in irritable bowel syndrome. Aliment Pharmacol Ther 2004; 2(Suppl 2): 1-9. [CrossRef]

11. Barkan AL, Dimaraki EV, Jessup SK, Symons KV, Ermolenko M, Jaffe CA. Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. J Clin Endocrinol Metab 2003; 88: 2180-4. [CrossRef]

12. Purnell JQ, Weigle DS, Breen P, Cummings DE. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J Clin Endocrinol Metab 2003; 88: 5747-52. [CrossRef]

13. Chan JL, Bullen J, Lee JH, Yiannakouris N, Mantzoros CS. Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. J Clin Endocrinol Metab 2004; 89: 335-43. [CrossRef]

14. Masaoka T, Suzuki H, Hosoda H, et al. Enhanced plasma ghrelin levels in rats with streptozotocin-induced diabetes. FEBS Lett 2003; 541: 64-8. [CrossRef]

15. Russo F, Chimienti G, Linsalata M, Clemente C, Orlando A, Riezzo G. The obstatin/ ghrelin ratio and ghrelin genetics in adult celiac patients before and after a gluten-free diet in irritable bowel syndrome patients and healthy individuals. Eur J Gastroenterol Hepatol 2017; 29: 160-8. [CrossRef]

16. Sjölund K, Ekman R, Wierup N. Covariation of plasma ghrelin and motilin in irritable bowel syndrome. Peptides 2010; 31: 1109-12. [CrossRef]

17. El-Salhy M, Lillebo E, Reinemo A, Salmelid L. Ghrelin in patients with irritable bowel syndrome. Int J Mol Med 2009; 23: 703-7. [CrossRef]

18. El-Salhy M, Gilja OH, Gundersen D, Hausken T. Endocrine cells in the oxyntic mucosa of the stomach in patients with irritable bowel syndrome. World J Gastrointest Endosc 2014; 6: 176-85. [CrossRef]

19. Tebbe JJ, Mronga S, Tebbe CG, Ortmann E, Arnold R, Schäfer MK. Ghrelin-induced stimulation of colonic propulsion is dependent on hypothalamic neuropeptide Y1- and corticotrophin-releasing factor 1 receptor activation. J Neuroendocrinol 2005; 17: 570-6. [CrossRef]