

# Gastric Histopathological Findings and Ghrelin Expression in Morbid Obesity

## Morbid Obezitede Gastrik Histopatolojik Bulgular ve Ghrelin Ekspresyonu

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### ABSTRACT

**Objective:** The role of Ghrelin, also known as the appetite hormone, is not fully explained in the development of morbid obesity. Plasma Ghrelin level is low in obese and high in slim subjects. Ghrelin-expressing cells were investigated histopathologically in the stomach of morbid obese patients in this study. Tissue Ghrelin expression was also compared with various parameters such as the distribution of endocrine cells, age, gender, body mass index, preoperative plasma Ghrelin level and presence of accompanying diseases.

**Material and Method:** The study included 33 morbidly obese patients, and 8 non-obese control patients. Plasma Ghrelin levels were measured preoperatively. Sleeve gastrectomy resection materials of 33 cases were evaluated with histopathological and immunohistochemical (Ghrelin and Chromogranin-A) techniques. The results were statistically evaluated by nonparametric tests.

**Results:** Histopathological findings observed in sleeve gastrectomy resection materials were interstitial lymphocytic infiltration (63.6%), hyperplasia of lymphoid follicles in the lamina propria (60.7%) and microvesiculation / dilatation of parietal cells (57.6%). The number of Ghrelin immunopositive cells in the gastric mucosa in females was significantly higher compared to males ( $p=0,007$ ). Additionally, the number of Ghrelin immunopositive cells was significantly higher at the fundus-proximal corpus compared to the distal corpus of the stomach ( $p=0,0001$ ). No significant correlation was found between Ghrelin-chromogranin immunopositive endocrine cell distribution and preoperative plasma Ghrelin levels and endocrine cell hyperplasia.

**Conclusion:** Our study confirms that Ghrelin producing cells are most dense in the proximal stomach. Increased number of Ghrelin expressing cells in the gastric mucosa in females compared to males suggests that gender may also be a factor in determining the method for treatment of morbid obesity.

**Key Words:** Morbid obesity, Ghrelin, Stomach

### ÖZ

**Amaç:** İştah hormonu olarak da bilinen Ghrelin hormonunun morbid obezite gelişimindeki rolü tam olarak açıklanamamıştır. Plazma Ghrelin düzeyi obezlerde düşük, zayıflarda yüksektir. Bu çalışmada; morbid obezitede midede görülen histopatolojik değişiklikler, Ghrelin eksprese eden hücreler araştırılmış, doku Ghrelin ekspresyonu; doku endokrin hücre dağılımı, yaş, cinsiyet, vücut kitle indeksi, preoperatif serum Ghrelin düzeyi ve eşlik eden hastalık varlığı gibi parametrelerle karşılaştırılmıştır.

**Gereç ve Yöntem:** Çalışmaya morbid obezite nedeniyle laparoskopik "sleeve" gastrektomi uygulanan 33 olgu ve obez olmayan 8 kontrol hastasının mide rezeksiyon materyalleri dâhil edildi. Olguların preoperatif plazma Ghrelin düzeyleri ölçüldü. Sleeve gastrektomi materyalleri prospektif olarak histopatolojik ve immünohistokimyasal yöntemlerle (Ghrelin ve Kromogranin-A) değerlendirildi. Bulgular istatistiksel olarak nonparametrik testlerle karşılaştırıldı.

**Bulgular:** Sleeve gastrektomi materyallerindeki histopatolojik bulgular; interstisyel lenfosit infiltrasyonu (%63,6), lamina propriada lenfoid folikül hiperplazisi (%60,7) ve pariyetal hücrelerde mikrovezikülasyon/dilatasyon (%57,6) olarak saptandı. Kadınlarda gastrik mukozadaki Ghrelin immünopozitif hücre sayısı erkeklere kıyasla anlamlı düzeyde yüksekti ( $p=0,007$ ). Ayrıca Ghrelin immünopozitif hücre sayısının, midenin fundus-proksimal korpus kısmında distal korpusa göre anlamlı olarak yüksek olduğu gözlemlendi ( $p=0,0001$ ). Ghrelin ekspresyonu ile kromogranin-A immünopozitif endokrin hücre dağılımı, araştırılan diğer parametreler veya preoperatif plazma Ghrelin düzeyi ile endokrin hücre hiperplazisi arasında anlamlı ilişki saptanmadı.

**Sonuç:** Çalışmamız, Ghrelin üreten hücrelerin midenin proksimalinde daha yoğun olduğunu doğrulamaktadır. Kadınlarda gastrik mukozadaki Ghrelin eksprese eden hücre sayısının erkeklerden yüksek olması ise morbid obezite tedavisi için seçilecek yöntemde cinsiyet faktörünün de etkili olabileceğini düşündürmektedir.

**Anahtar Sözcükler:** Morbid obezite, Ghrelin, Mide

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## INTRODUCTION

Morbid obesity defines a severe obesity that is life-threatening and shortens the person's life where the body mass index (BMI) is over 40. The most important factor responsible for the development of morbid obesity is the energy balance being distorted in favor of food intake and storage (1). Ghrelin is one of the hormones considered to have the greatest impact on the energy balance.

Ghrelin, which is also known as the appetite hormone, was discovered by the Japanese scientist Masayasu Kojima in 1999 and is a molecule acting on many systems and thought to have an important role in the development of obesity (2,3). However, how the hormone ghrelin affects the development of morbid obesity is not yet known.

The aim of this study was to determine the histopathological changes seen in the stomach in morbid obesity, to evaluate the distribution of endocrine cells and ghrelin expressing cells in the stomach of these patients, and to examine the relationship between the plasma ghrelin levels and ghrelin immunoreactive cell density.

## MATERIAL and METHODS

Gastric resection materials of 33 cases (24 female, 9 male) who underwent laparoscopic "sleeve" gastrectomy (LSG), also known as a "stomach tube" operation, with a diagnosis of morbid obesity at the Pamukkale University Department of General Surgery, Faculty of Medicine between 2010 and 2011 were prospectively evaluated with histopathological and immunohistochemical methods. In addition, 8 (5 males, 3 females) patients who were not "obese/morbidly obese" according to BMI and had undergone partial/total gastrectomy because of reasons other than obesity were included in the study as a control group for comparison with the patient group in terms of tissue ghrelin and Chromogranin-A expression and blood ghrelin levels.

The data such as age, gender, weight, height, BMI, and presence of additional disease (diabetes, hypertension, heart disease) in the study group were obtained from the clinical files.

Blood samples for the plasma ghrelin level were obtained just before the operation and the measurements were performed at the Medical Biochemistry Department with a commercial Elisa method and kit (Ghrelin Enzyme Immunoassay Kit, Code: ANNEX-031-30, Phoenix Pharmaceuticals, INC.).

Resection materials were fixed with 10% buffered neutral formalin for 16-24 hours. On the fixated LSG material,

a total of eight samples from the standard anatomical locations (three from the proximal and middle of the resection specimen and two from the distal) were taken from each patient. The samples taken from the proximal end (fundus) and middle (proximal corpus) of the resection material were coded as "proximal" while the samples taken from the distal end (distal corpus) were coded as "distal" samples. The samples obtained were cut 4 µm thick after the routine tissue follow-up procedure and stained with Hematoxylin-Eosin (H&E) to be ready for microscopic examination. The presence of lymphoid follicle hyperplasia, interstitial lymphocytic infiltration and congestion in the lamina propria, erosion/ulceration in the mucous surface epithelium, microvesiculation/dilatation and neutrophilic infiltration in parietal cells and dilated gland structure in the mucosa were evaluated in detail in the proximal and distal samples. Lymphoid follicle hyperplasia in lamina propria, interstitial lymphocytic infiltration and congestion parameters were graded semiquantitatively between 0 and 3 as (0) absent/normal range, (1) mild, (2) moderate, and (3) severe. The evaluation was done separately for the proximal and distal corpus. Other histological parameters (erosion/ulceration in the mucosal surface epithelium, microvesiculation/dilation and neutrophilic infiltration in parietal cells, dilated gland structures in the mucosa) and other clinical parameters (associated diseases such as diabetes, hypertension, heart disease) were evaluated as "yes/no" for each patient.

Two blocks, one each from the proximal and distal coded samples, were selected for each patient for immunohistochemical examination. 3-micron thick sections were prepared from the selected paraffin blocks for Ghrelin and endocrine cell determinant (Chromogranin-A) immunohistochemical analysis and the procedure was performed on a fully automated immunohistochemistry device (VENTANA Benchmark / LT, Ventana Medical Systems, USA). The sections were applied Ghrelin monoclonal antibody (Abcam, Cambridge, United Kingdom, Code: ab57222, dilution: 1/200, retrieval EDTA 60 min, 30 min. incubation) and Chromogranin-A monoclonal antibody (Thermo Scientific, USA, Clone: LK2H10, dilution: 1/800, retrieval EDTA 60 min, 32 min. incubation) drops as the primary antibody to visualize the targeted proteins. The immunohistochemically stained sections were evaluated with the method below.

**Ghrelin:** The evaluation was performed separately for proximal and distal coded samples in each case. On light microscope (Olympus BX51) at x10 magnification, Ghrelin-immunopositive cells were counted in 5 most appropri-

ate areas containing the mucosa in full-thickness from the surface of the stomach mucosa to the muscularis mucosa. The total number was divided by five, and the arithmetic means for both proximal and distal segments were taken. In addition, the numerical data obtained for the proximal and distal parts were added and divided into two and the mean Ghrelin immunopositive cell number in each case was obtained.

**Chromogranin-A:** The evaluation was performed separately for the proximal and distal coded samples in each case. On light microscope (Olympus BX51) at x10 magnification, chromogranin immunopositive cells were counted in 2 most appropriate area containing the mucosa in full-thickness from the surface of the stomach mucosa to the lamina muscularis mucosa. The total number was divided by two, and the arithmetic means for both proximal and distal segments were taken. Cases displaying too much staining to count with Chromogranin-A were also assessed in terms of endocrine cell hyperplasia (focal simple hyperplasia, simple hyperplasia, micronodular hyperplasia) and were graded in five categories as follows:

- 0: Within normal limits (between 0-199 chromogranin immunopositive cells)
- 1: Mildly increased (between 200-300 chromogranin immunopositive cells)
- 2: Focal simple hyperplasia
- 3: Simple hyperplasia
- 4: Micronodular hyperplasia

SPSS software (version 10.0) running on a computer environment was used for all statistical analyzes. The  $\chi^2$  (chi-square) test was used for non parametric evaluations, and the t-test in independent groups, Wilcoxon and Mann-Whitney U tests were used for other evaluations. A p value <0.05 was accepted as significant for the obtained results.

## RESULTS

A total of 33 morbid obese patients (24 females, 9 males) and 8 control patients (5 males, 3 females) who were not obese were included in the study. The mean age of the morbid obese patients were  $35.90 \pm 9.53$  (18-60) years, and the preoperative mean plasma ghrelin level was  $67.21 \pm 14.52$  (20.2-92.4) ng/ml. The mean plasma ghrelin level in the control group was  $53.1 \pm 15.51$  ng/ml.

The median weight of the obese patient group was  $134.96 \pm 17.86$  (between 106-178) kg, the median height  $165.09 \pm 8.36$  (150-185) cm and the median BMI  $49.75 \pm 7.28$

(36-68 kg/m) kg/m<sup>2</sup>. There was no morbidly obese subject in the control group according to BMI. In terms of additional disease, 8 of the 33 obese patients (24.2%) had concurrent hypertension (HT), 9 (27.3%) had diabetes mellitus (DM), and 2 (6.1%) had coronary artery disease.

As a result of histopathological examination of LSG materials of the cases, 23 (69.7%) were evaluated as chronic gastritis, 3 as erosive gastritis (9.1%), 2 (6.1%) as chronic active gastritis, 1 as congested stomach tissue (3%) and 4 as normal stomach tissue (12.1%). Clinical characteristics of the patients involved in the study are summarized in Table I and the histopathological findings in Figure 1A-D.

The distribution of the histopathological lesions we observed in LSG resection materials were microvesiculation/dilatation in parietal cells in 57.6%, erosion/ulceration in the mucosal surface epithelium in 57.6%, dilated gland structure in the mucosa in 57.6% and neutrophilic infiltration in 6.1%. In addition, moderate-to-severe lymphoid follicle hyperplasia was detected in the lamina propria in 20 patients (60.7%), interstitial lymphocytic infiltration in 21 patients (63.6%) and mucosal congestion in 14 patients (42.4%). The presence of *Helicobacter pylori* was not investigated in resection materials. The distribution of observed histopathological findings showed no significant difference in the proximal and distal gastric corpus mucosa. No significant correlation was detected between the histopathological findings and the parameters evaluated in obese patients, other clinical findings, immunohistochemical findings and plasma Ghrelin levels.

When the number of immunopositive ghrelin cells was evaluated in the general population including the patient and control group (41 subjects in total), it was significantly higher in the proximal corpus part of the stomach than the distal corpus part (Wilcoxon test,  $p=0.0001$ , Figure 2). The number of Ghrelin immunopositive cells in the gastric mucosa in females was found to be significantly higher than males ( $73.14 \pm 23.71$  vs.  $54.57 \pm 16.51$ , Mann-Whitney U test,  $p=0.007$ , Table II).

There was no significant difference between the control group and the patient group in terms of the preoperative plasma Ghrelin levels, Ghrelin immunopositive cell numbers and the staining severity.

No significant relation was found between Ghrelin or Chromogranin-A immunopositive cell distribution or preoperative plasma Ghrelin level - endocrine cell hyperplasia. No statistically significant difference was found between the two genders in terms of the preoperative plasma Ghrelin level.

**Table I:** The clinical features of the patients

<b>Obese Patient Number</b>	Male	9 (27.3%)
	Female	24 (72.7%)
<b>Age (median ± SD)</b>		35.90±9.53 years
<b>Weight (median ± SD)</b>		134.96±17.86 kg
<b>Height (median ± SD)</b>		165.09±8.36 cm
<b>BMI (median ± SD)</b>		49.75±7.28 kg/m <sup>2</sup>
<b>Preoperative plasma Ghrelin level (median ± SD)</b>		67.21±14.52 ng/ml
<b>Pathological diagnosis N (%)</b>	Chronic gastritis	23 (69.7)
	Erosive gastritis	3 (9.1)
	Chronic active gastritis	2 (6.1)
	Congestioned stomach tissue	1 (3)
	Normal stomach tissue	4 (12.1)
<b>Diabetes Mellitus N (%)</b>	Yes	9 (27.3)
	No	24 (72.7)
<b>Hypertension N (%)</b>	Yes	8 (24.2)
	No	25 (75.8)
<b>Coronary artery disease N (%)</b>	Yes	2 (6.1)
	No	31 (93.9)

No significant relation was found between the presence of associated diseases (DM and HT) and the Ghrelin immunopositive cell number or preoperative plasma Ghrelin level.

### DISCUSSION

Obesity is now a major health problem with an impact on mortality and morbidity and compromises the sociocultural life of the person. It ranks second among deaths with an unknown cause. Bariatric surgery offers the best treatment for sustained weight loss in morbidly obese patients at present (4).

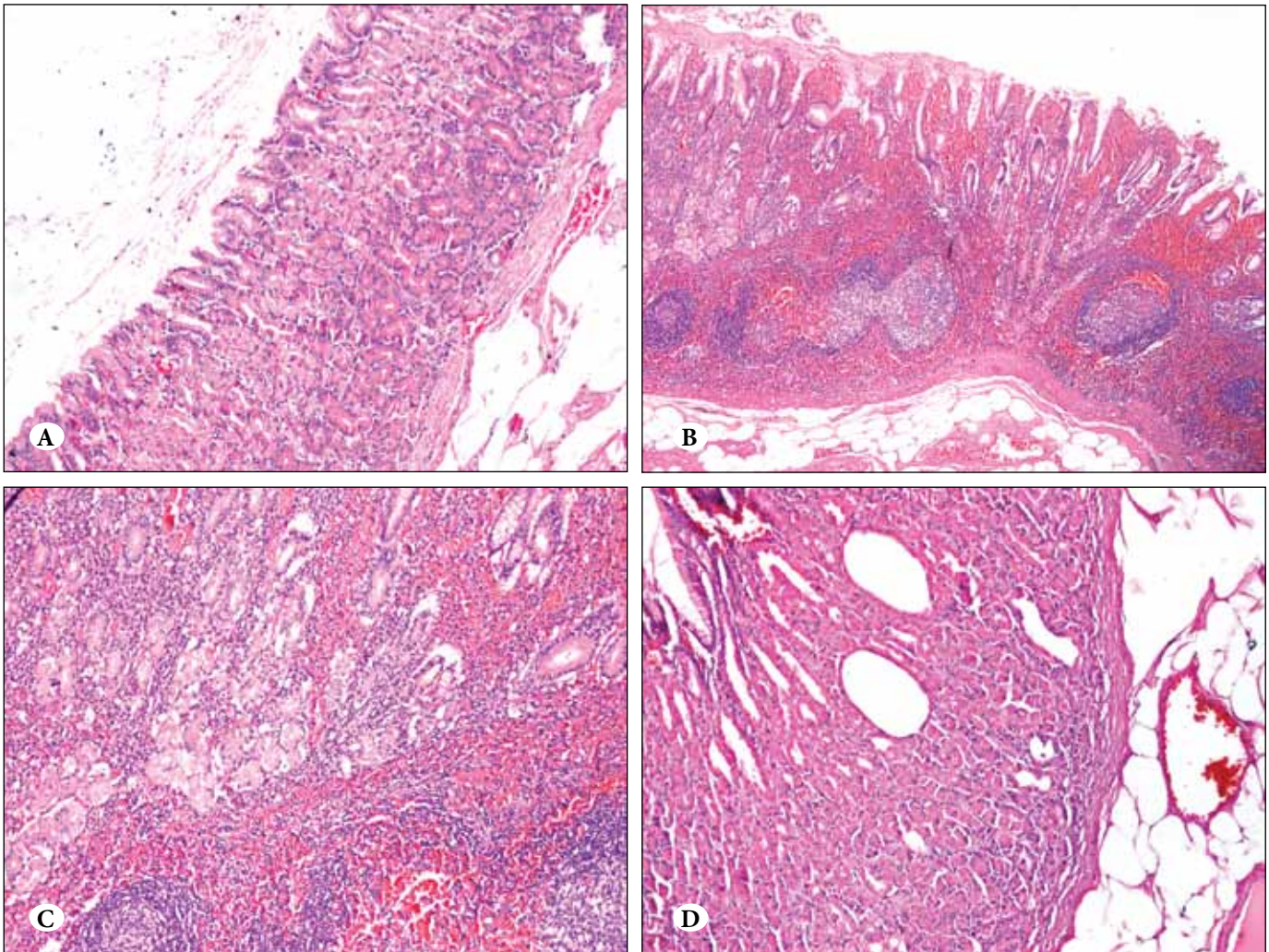
Many factors are responsible for the development of morbid obesity. However, the most important factor is the distortion of the energy balance in favor of nutrient intake and storage. Many organs, particularly the hypothalamus, take part in ensuring the balance and some specific peptides allow communication between these tissues. Ghrelin is one of the most important of these peptides.

Ghrelin is an orexigenic peptide that increases appetite and thus food intake, and is a hormone that has been found to contribute to maintaining the energy balance. Ghrelin was found in 1999 by the Japanese scientist Masayasu Kojima and his team that worked in the cardiovascular area and is mainly released from the fundus of the stomach (2). It also causes growth hormone secretion from pituitary and

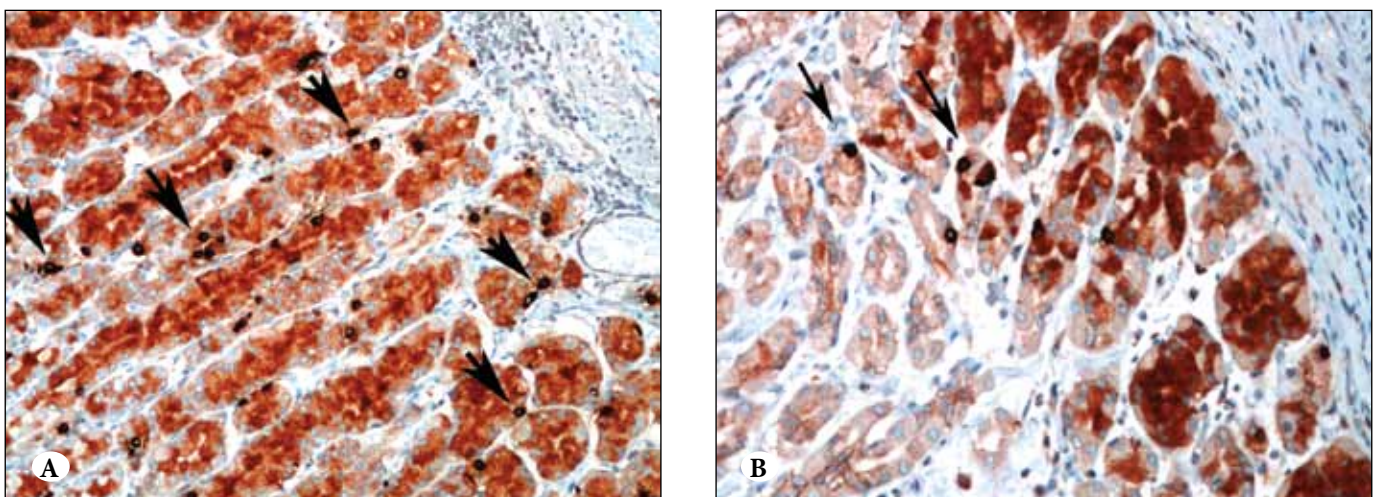
shows many of its physiological effects in this way. It is mainly effective on carbohydrate and energy metabolism, pancreatic exocrine and endocrine function, gastrointestinal system, eating and the cardiovascular system (2). It is thought to have autocrine/paracrine effects in many tumor tissues. Intravenous Ghrelin injection increases gastric acid secretion and gastric motility proportional to the dose. Ghrelin is thought to affect gastric functions via the vagus nerve.

Sakata et al. investigated Ghrelin-producing cells in the gastrointestinal system by the immunohistochemical method. The Ghrelin-immunopositive cells were detected to be mostly localized in the stomach and less in the duodenum, jejunum, ileum, cecum, and colon (6). Ghrelin is mainly synthesized in X/A-like cells that are specialized endocrine cells in the gastric oxyntic mucosa. These cells are localized in the full thickness of the oxyntic gastric mucosa and topographically correlate with parietal cells (5). Ghrelin immunopositive cells were seen only in the mucosal layer of the stomach, in the gastric glands of the lamina propria in our study. In a study by Stenstrom et al., the stomach section that contains the oxyntic mucosa was surgically removed in rats and the Ghrelin concentration in the circulation was found to be reduced by 80% (7).

In the gastrointestinal system, Ghrelin immunoreactive cell density decreases from proximal to distal. When we



**Figure 1:** Histopathological results in LSG materials. (A) Stomach corpus wall within normal limits (H&E, x100), (B) Chronic gastritis characterized by lymphoid hyperplasia (H&E, x100), (C) Interstitial lymphocytic infiltration between gastric glands (H&E, x200), (D) Micro-vesiculation/dilation in parietal cells (H&E, x100).



**Figure 2:** Ghrelin expression. (A) Increased number of Ghrelin immunopositive cells in the corpus mucosa of the proximal stomach (marked with bold arrows, x200), (B) Smaller number of Ghrelin immunopositive cells in distal corpus mucosa (marked with thin arrows, x200).

**Table II:** Ghrelin immunopositive cell distribution in females and males

	N	Ghrelin (+) cell number	p
Female	24	73.14±23.71	0.007
Male	9	54.57±16.51	0.007

analyzed a total of 41 cases, a significantly higher number of Ghrelin immunopositive cells were detected in the fundus-proximal corpus part of the stomach than the distal corpus part in our study, in conformance with other studies. Tanaka-Shintani and Watanabe took samples from the small curvature area of the stomach without a tumor in 30 patients with gastric cancer and examined the Ghrelin expression immunohistochemically. They found Ghrelin-immunoreactive cells in all parts of the stomach, but mostly in the fundus area (5). Lee et al. found the highest expression in terms of immunolocalization of Ghrelin in different areas in the fundus in rats (8).

The main regulator of the plasma Ghrelin level is food intake. The level rises in starvation and causes the person to eat, while it is suppressed after eating and the level falls. Oral or intravenous glucose administration decreases plasma Ghrelin levels (9,10). Sönmez and Ozan, in their study on rats, found the number of Ghrelin cells in the stomach in starvation to be increased compared to the control group; the number of cells showing immunoreactivity decreased and regressed to values close to the control group following re-feeding after starvation (11). The plasma Ghrelin level was not investigated in that study.

Although Ghrelin is known as the appetite hormone, plasma Ghrelin levels and the BMI interestingly show an inverse relationship. Ghrelin secretion was observed to be increased in anorexia and cachexia, decreased in obesity, and normal in ideal body weight (12).

In a study of Vrabie et al. with features similar to our study, the main histopathological gastric lesions in obese patients who had undergone LSG were investigated. The majority of patients, as in our study group, were reported to be young and female. Gastric resection material was obtained from 87 surgical interventions where LSG was performed between 2007 and 2008, and the most common histological microscopic lesions were parietal cell hyperplasia (63.2%), ulceration (34.5%), lymph node hyperplasia in the lamina propria (33.3%), active gastritis (23%), and other lesions (10.3%) (13). We have found no hyperplasia in the parietal cells but microvesiculation and dilatation were observed.

The LSG operation “suppresses” Ghrelin levels and thereby reduces appetite and increases weight loss in obese patients.

Wang and Liu, in the study they conducted by using the clinical data of obese patients who underwent LSG, determined that the patients lost 60% of their excess weight in the 2-year follow-up (14).

A number of studies that report various findings and results about the relationship of plasma Ghrelin level with age, gender, and BMI are present in the literature. We found no statistically significant relationship between ghrelin immunopositive cell numbers and the age of the patient, BMI or plasma Ghrelin levels. On the other hand, we also could not find any statistically significant relationship between the preoperative plasma Ghrelin level and gender.

However, the number of Ghrelin immunopositive cells in the gastric mucosa in females were significantly higher than males in our study when we evaluated a total of 41 patients. There is no similar study previously conducted in the literature regarding this situation we determined so we think this result is significant in terms of the morbid obesity treatment method choice.

Purnell et al. reported a positive relation between starvation Ghrelin level and age similar to our study in a study they conducted in a population consisting of 60 adult males and females with a wide age and weight range. However, unlike our result, they found the fasting Ghrelin level to be negatively related to the BMI, to be similar in males and females and not change with the menopausal status (15).

Zou et al. reported the plasma Ghrelin level to show negative correlation with BMI and have no relation with factors such as age and gender in their study on 283 subjects (16).

Chuang et al. found plasma Ghrelin levels to be similar in males and females in cases not accompanied by HP infection in their study on 341 patients with dyspepsia. Among patients positive for HP, the plasma Ghrelin levels were found to be lower in males. In addition, a strong correlation was found between HP infection and acute and chronic inflammation scores associated with it and lower Ghrelin levels but only in males (17). Our chromogranin-A immunohistochemical analysis performed to evaluate the distribution of endocrine cells in LSG resection material revealed a mild increase in endocrine cells in 13 patients (39.4%), focal simple hyperplasia in 3 patients (9.1%),

simple hyperplasia in 7 patients (21.2%) and micronodular hyperplasia in 3 patients (9.1%). Seven patients (21.2%) were considered to be within the normal range of endocrine cell density. With these findings, a significant relation was found neither between Ghrelin and chromogranin-A immunopositive endocrine cell distribution nor between the preoperative plasma Ghrelin level and endocrine cell hyperplasia. Our results in this respect were consistent with several previous studies in the literature (18-20).

We found Ghrelin immunopositive cell number to be significantly lower in 9 obese patients with DM than in patients without DM, similar to the study of Maghsoud et al. (21). In addition, although the preoperative plasma Ghrelin level was found to be higher in non-diabetic patients, the difference was not statistically significant.

Pöykkö et al. obtained blood samples from 1040 subjects in their study to determine whether low blood Ghrelin concentration was a risk factor for type 2 DM. They found low Ghrelin levels related to type 2 DM, high insulin concentration, insulin resistance and increased blood pressure (22).

Bideci et al. compared the plasma Ghrelin levels of 27 child patients with type 1 diabetes and 25 healthy children. They reported the Ghrelin levels to be similar and normal in the control group and the group of patients with type 1 diabetes (23).

There are various opinions on the relationship between plasma Ghrelin concentrations and HT in the literature. Although we found the preoperative plasma Ghrelin level to be higher in patients with HT than non-HT patients, the difference was not statistically significant. In the previously mentioned study of Pöykkö et al., low blood ghrelin concentration was reported to be a risk factor for HT and ghrelin levels were negatively associated with systolic and diastolic blood pressure. Thus, they argued that Ghrelin may have a role in type 2 DM etiology and regulation of blood pressure (22). İyidoğan et al. reported Ghrelin to be positively associated with HT in obese women and an inverse relationship developed with the increase in BMI (24).

LSG is an important obesity surgery which is effective in a short time and with growing popularity in obesity surgery. The superior clinical results with LSG have focused attention on the partial gastric resection option and possible effects of mediators released from the resected portion. The role of the Ghrelin hormone in the development of morbid obesity is not yet known. However, we think that especially the Ghrelin-producing fundus area is removed

with LSG, and we can say that Ghrelin is a hormone closely linked with obesity taking into account the marked loss in appetite in the period after LSG. The higher number of cells expressing Ghrelin in the proximal of stomach in our study is consistent with the results of other relevant studies. In addition, there is no similar study in the literature reporting the detection of a higher number of cells expressing Ghrelin in the gastric mucosa in females than in males. We believe this result may be important in the choice of treatment of morbid obesity. However, new studies are required to prove this hypothesis.

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