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Original article

Comparison of the results of sleeve gastrectomy, gastric pilication and liragulitide in obese rats

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Abstract

Obesity, which is generally seen in adults, is a serious health problem. Diseases caused by obesity are among the leading causes of death worldwide. Liraglutide (LG) is an analogue of glucagon-like peptide-1, which slows gastrointestinal motility, resulting in decreased food consumption. Gastric plication (GP) and sleeve gastrectomy (SG) is the reduction of stomach volume by surgical means. We examined and compared the body mass index (BMI) changes, metabolic changes and changes in gastric histology in obese rats after LG injection with surgical methods such as SG and GP.

In this research, 35 Wistar Albino female rats were used. Rats were divided into 5 groups with 7 rats in each group. Group (G) 1: The control group, fed with a normal calorie diet for 8 weeks. G 2: Sham group, G 3: SG group, G 4: GP group and G 5: LG group, fed with high-calorie feed for 4 weeks. At the end of the 4th week, the study was terminated by making appropriate interventions for the groups.

When the blood glucose (BG) levels measured at the beginning, 4th week and 8th week of the experiment were evaluated, it was monitored that the BG level at the 8th week was the lowest in the LG group ($p<0.05$). It was observed that the preop Ghrelin and Leptin levels of the LG group were lower than those of the SG and GP groups ($p<0.05$).

As a consequence of our metabolic investigations, we observed that the use of LG is at least as effective as SG.

Keywords: obesity, liraglutide, sleeve gastrectomy, gastric plication

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Introduction

Obesity, which is one of the most serious health issues particularly for adult individuals, is the fifth leading reason of death worldwide (Dag et al. 2015). Mortality rate in obese population is 20% higher than in normal weight people (Borrell et al. 2014). In obese people, lipid metabolism is disrupted and excessive fat accumulation occurs in the body (Masarone et al. 2014). The World Health Organization (WHO) determines obesity qua a body mass index (BMI) equivalent to or greater than 30 kg/m² (Dag et al. 2015).

Liraglutide (LG) is secreted in response to carbohydrate and fat absorbed from the ileum. It has various physiological effects in the body. It suppresses glucagon release while triggering insulin release, lowering the blood sugar level and thus balancing the glucose homeostasis. It also slows down gastrointestinal motility, resulting in decreased food consumption (Sturis et al. 2003). Studies have shown that Liraglutide use reduces the risk factors of obesity by reducing waist circumference (Astrup et al. 2009).

Laparoscopic gastric plication (GP) and laparoscopic sleeve gastrectomy (SG) are bariatric surgical techniques based on the anatomical principles. The GP technique involves reducing the volume of the stomach by invagination, thereby reducing food intake. However, its effects on metabolism are not yet fully known. Clinical practice of GP is not common (Talebpour et al. 2017). SG, on the other hand, provides resection along the curvature of the stomach to reduce the volume of the stomach (Shi et al. 2010). On the other hand, SG, which was initially considered a limiting technique after the publication a lot of studies, has been used as a metabolic component (Talebpour et al. 2017).

In this study, we tried to determine which of these methods is more advantageous by examining and comparing the changes in metabolic profile and gastric histology of rats after Liraglutide injection as well as surgical procedures such as SG and GP in obese rats.

Materials and Methods

High-fat diet preparation

After grinding the pellets purchased from a commercial company producing feed for rats, 300 g of butter was melted into 1000 g of feed and mixed to obtain high calorie feed (4165 kcal/kg) (Gunbatar et al. 2015). Rats to be obese were fed with high calorie feeds. The rats in the control group were fed with normal calorie (1920 Kcal/kg) pellets.

Experimental procedures

Sleeve gastrectomy procedure

After 12 hours of fasting, the rats were placed under general anesthesia. 90 mg/kg Ketamine (Ketalar®, Pfizer) + 10 mg/kg Xylazine hydrochloride (Basilazine 25 ml 2% vial, Bayer, Germany) was applied and the hairs in the abdominal region were shaved and cleaned. 100 mg/kg Cefazolin Sodium intramuscularly (i.m.) was administered prophylactically. After the abdominal skin was cleaned with povidone iodine, a laparotomy was performed with a 4 cm long incision from the midline, and the stomach was defined, and sleeve gastrectomy was maked in a longitudinal plane, starting from the pylorus and extending to the esophago-cardiac junction appropriately. The remainder of the stomach was double sealed with 5/0 prolene. The operation was terminated by closing the subcutaneous tissue and the skin. Postoperative analgesia was provided by peroral administration of 20 mg/kg (p.o.) paracetamol and daily dressing was done (Marta et al. 2013).

Gastric plication procedure

After applying a similar surgical protocol, 5/0 prolene plication was performed from the pylorus to the esophago-cardiac junction (Marta et al. 2013).

Liraglutide application

Liraglutide used in the study was obtained from Novo Nordisk (Copenhagen, Denmark) and stored at +4°C. The therapeutic dose of liragulutide daily for rats to be administered liraglutide was: First Week: 0.6 mg/ml (0.06 mg/kg) s.c., Second Week: 1.2 mg/ml (0.12 mg/kg) s.c., Third Week: 1.8 mg/ml (0.18 mg/kg) s.c., Week Four: 2.4 mg/ml (0.24 mg/kg) s.c. (Saber et al. 2019).

Formation of experimental groups

Ethics committee approval for this study was receipted from Dicle University Local Ethics Committee with protocol number 2021/20. A total of 35 female Wistar Albino rats were utilizated in the study. To induce obesity, animals were fed high-calorie pellet feeds for 4 weeks (Gunbatar et al. 2015).

Group 1 (n=7): The control group was given normal calorie feed during the study period,

Group 2 (n=7): Sham group received high calorie feed for 4 weeks tracked by 4 weeks of normal calorie feed.

Group 3 (n=7): The rats in the SG group were also given high-calorie diets for 4 weeks. After 4 weeks, their body mass index (BMI) was calculated and rats

Table 1. Mean±standard deviation values of baseline, week 4 and week 8 blood glucose and body mass index (BMI) in rats.

Groups	BG Start (mg/dl)	BG (week 4) (mg/dl)	BG (week 8) (mg/dl)	BMI Start (kg/m^2)	BMI (4. week) (kg/m^2)	BMI (8. week) (kg/m^2)
Control	101.14±7.24	113.14±9.65^{b,c,d,e}	111.57±10.51^{b}	3.08±0.36	3.28±0.26^{b,c,d,e}	3.79±0.20^{b}
Sham	101.57±7.69	157.00±10.55^{a}	150.71±12.45$^{a,\ e}$	3.63±0.24	5.15±0.10^{a,}	4.66±0.35^{a}
SG	105.00±15.62	168.71±11.36^{a}	129.28±9.55	3.64±0.12	5.42±0.23^{a}	3.82±0.41
GP	98.57±5.09	178.42±17.15^{a}	126.28±15.11	3.34±0.48	5.47±0.27^{a}	4.18±0.14
LG	96.71±5.90	170.71±13.68^{a}	108.57±14.61	3.61±0.29	5.46±0.24^{a}	3.88±0.49

BG – Blood Glucose. BMI – Body Mass Index, SG – sleeve gastrectomy, GP – gastric plication, LG – Liraglutid. a – there is a difference with the control, b – there is a difference with sham, c – there is a difference with SG, d – there is a difference with GP, e – there is a difference with LG ($p<0.05$).

over 5 kg/m^2 were considered obese and SG was performed and then fed with normal calories.

Group 4 (n=7): The GP group underwent similar procedures as SG and underwent GP at the lasted of the 4th week.

Group 5 (n=7): In the LG group, the therapeutic dose of LG was administered subcutaneously (s.c.) at the lasted of the 4th week without any surgical procedure.

The rats were sacrificed by exsanguination under general anesthesia at the end of the experiment. Blood samples were centrifuged at 3000 rpm for 10 minutes and serum samples were taken and then Gastrin, Leptin and Ghrelin hormone levels were analyzed with ELISA kits. Gastric tissues were placed in 10% formol and sent to Histology laboratory.

Blood glucose measurements

Blood glucose values were measured and recorded from the blood samples taken from the jugular vein on the first day, 4th week and at the lasted of the experiment.

Calculation of BMI

Before starting the study, the rats were measured from the type of the nose to the tip of the tail to establish their height. Body weights were also noted before starting the research. At the last of the 4th week, rats with a BMI of 5 kg/m^2 and above in the study groups fed with high-calorie feeds were considered obese (Altunkaynak et al. 2008).

Gastrin, ghrelin, and leptin hormone levels

At weeks 4 and 8, after BMIs were calculated, blood samples obtained from the jugular vein were analyzed for Gastrin, Ghrelin and Leptin hormone levels. ELISA kits which were purchased from Shanghai Eugene Biotech Co. Ltd, China, were used to evaluate hormone levels. Thus, pre-operative and post-operative changes of three hormones were examined.

Histopathological examinations

The gastric tissues were fixed in 10% formol for 24 hours and subjected to routine histologic follow-up. Sections of 4 µm thick gastric tissues embedded in paraffin blocks were stained with Hematoxylin & Eosin (H&E) stain to evaluate the levels of fibrosis. Damage score was determined as 0=none, 1=mild, 2=moderate, 3=severe.

Statistical analysis

The numerical values obtained were analyzed using SPSS 20 software (Chicago, IL, USA). Kruskal Wallis-H test was performed for normally distributed values, while Mann Whitney-U test was used for intergroup comparison ($p<0.05$ significant).

Results

Blood glucose and body mass indices measurements

When the blood glucose levels of the groups measured at the beginning, 4th week and 8th week of the experiment were compared, Kruskal Wallis-H test of the initial blood glucose levels was not significant, so no comparison was made between the groups ($p>0.05$). In the 4th week blood sugar levels analysis, it was found that there was a significant discrimination between the control group and the other study groups, with the control group having the lowest mean ($p<0.05$). It was found that there was no significant discrimination between the blood sugar levels of the SG, GP, LG and sham groups ($p>0.05$).

When 8th week blood glucose levels were checked, it was found that there was no significant difference between the SG group and the LG group ($p>0.05$), and the lowest blood glucose mean was observed in the LG group. However, no statistically significant difference was found between the GP group and the LG group ($p>0.05$) (Table 1).

Table 2. Mean±standard deviation values of Preop and Postop gastrin, ghrelin and leptin levels in rats.

Groups	Gastrin (ng/ml) (preop)	Gastrin (ng/ml) (postop)	Ghrelin (ng/ml) (preop)	Ghrelin (ng/ml) (postop)	Leptin (ng/ml) (preop)	Leptin (ng/ml) (postop)
Control	20.88±1.39[b,c,d,e]	21.04±1.33[b,c,d,e]	5.99±0.54[b,c,d,e]	5.68±0.56[b,c,d]	1.25±0.15[b,c,d,e]	1.19±0.09
Sham	26.72±2.11[a]	27.29±2.13[a]	9.68±0.87[a]	8.43±0.49[a]	2.45±0.44[a]	1.81±0.44
SG	25.90±2.23[a]	28.85±2.41[a]	10.38±0.71[a]	7.58±0.98[a]	2.09±0.35[a]	1.13±0.13
GP	25.48±2.86[a]	26.76±2.51[a]	9.27±1.08[a]	8.35±0.62[a,e]	2.31±0.42[a]	1.35±0.31
LG	26.67±2.44[a]	29.04±2.85[a]	9.65±1.06[a]	6.55±1.12	2.35±0.42[a]	1.31±0.19

SG – sleeve gastrectomy, GP – gastric plication, LG – Liraglutid. a – There is a difference with the control, b – There is a difference with sham, c – There is a difference with SG, d – There is a difference with GP, e – There is a difference with LG ($p<0.05$).

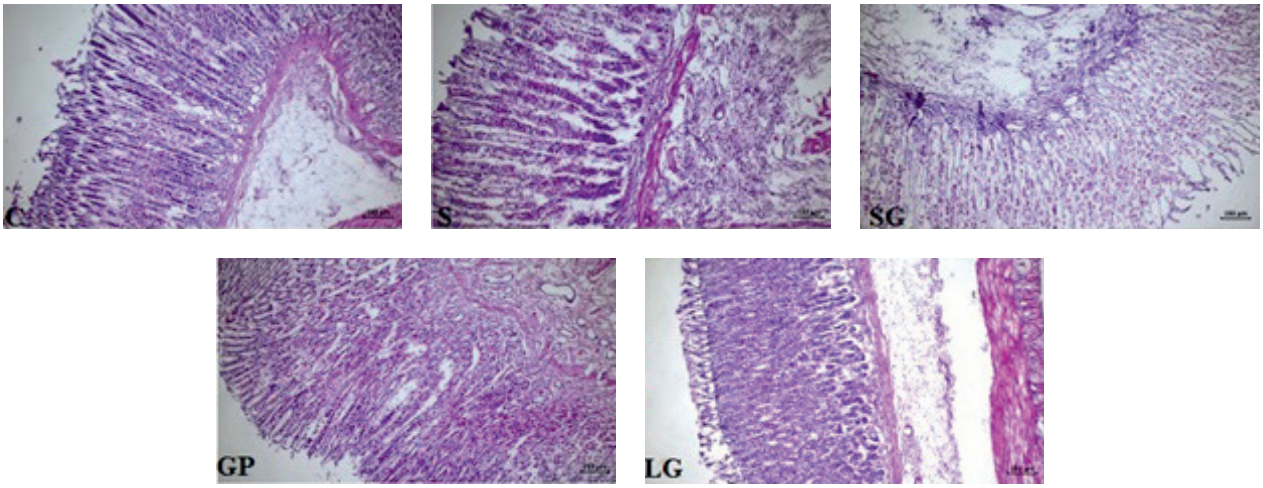

Fig. 1. C – Normal histologic appearance of gastric tissue in control group, S – Normal histologic appearance of gastric tissue in sham group, SG – Histologic appearance of gastric tissue after sleeve gastrectomy, GP – Histologic appearance of gastric tissue after gastric plication, LG – Histologic appearance of gastric tissue of liraglutide group (Hematoxylin and eosin stain, Bar = 100 µm).

Similarly, when the calculated BMIs were analyzed, no significant difference was found between the baseline BMIs of all study groups ($p>0.05$). At week 4, when we compared the groups, the BMI of the control group was lower than the other study groups ($p<0.05$). At week 8, when we compared the BMIs, there was no statistically significant difference between the SG group and the LG group ($p>0.05$). It was revealed that the SG group had the lowest mean, while the SG group ranked second (Table 1).

Gastrin, ghrelin, and leptin hormone levels

Gastrin hormone level was lower in the control group than in the other groups in both preop and postop periods ($p<0.05$), while there was no significant difference between the other groups ($p>0.05$). Gastrin hormone level was not affected by surgical methods and LG injection.

In the preop and postop comparison of ghrelin hormone levels between the groups; ghrelin hormone levels of all study groups were higher than the control group in the preop period ($p<0.05$). When we checked the ghrelin hormone levels of the groups in the postop period, there was no significant difference between the control group and the LG group ($p>0.05$). The LG injection we administered had a positive effect on ghrelin hormone level in the postoperative period and ensured that it had a normal average as much as the control group.

In the comparison of leptin levels between the groups in the preop period, it was found that there was a statistically significant difference between the control group and the other study groups, with the lowest mean in the control group ($p<0.05$). There was no statistically significant difference between the groups in the postop period ($p>0.05$). While the mean leptin levels of all groups were almost the same, the lowest mean was found in the SG group and the LG group was very close to the SG group (Table 2).

Histopathological examinations

When the gastric tissues taken at the end of the experiment were examined, it was found that there was a raise in the levels of fibrosis in the gastric tissues

of SG and GP groups, while no histopathological changes were observed in the other study groups (Fig. 1).

Discussion

Obesity is known as a major health problem worldwide. Obesity is defined as excessive accumulation of fat tissue in the body. WHO defines obesity as BMI 40 kg/m^2 and above (Ewing et al. 2011, Domienik-Karlowicz et al. 2015). Apart from diet, medication and surgical methods might be applied in the treatment of obesity (López-Nava et al. 2015). Various studies are being carried out around the world for the treatment of obesity, which has been a major problem up to date. Laparoscopic sleeve gastrectomy (SG) is one of the most often used treatment methods recently. SG is a frequently used method with a low mortality rate and morbidity (Hirth et al. 2015).

In this study, we compared both the metabolic efficiency and histopathologic results of surgical methods (SG and GP), which are greatly utilizated in the treatment of obesity, and LG, which may be utilizated as an option.

With the discovery of leptin hormone, positive results have been obtained in the treatment of obesity and related diseases (Gulcicek et al. 2016). Leptin hormone is produced in fat cells in the body and released into the blood and sends satiety signals to the brain, thus keeping appetite under control (Tadokoro et al. 2015). Studies have shown that leptin hormone levels of individuals diagnosed with Anorexia nervosa are lower than in normal individuals (Gulcicek et al. 2016). In this research, we monitored that leptin hormone levels decreased as the BMI of obese rats decreased. Similar results to these studies were obtained in our study.

The hormone gastrin is released by G cells that stimulate gastric acid secretion. It is also a hormone that stimulates gastric and intestinal motility (Zhou et al. 2014). Gulcicek et al. observed an increase in gastrin hormone levels in the SG and GP groups in the postoperative period in their study (Gulcicek et al. 2016), whereas a decrease in postoperative gastrin hormone levels was observed in our study. In this context, our study differed from other studies conducted.

The hormone ghrelin is a peptide hormone that affects appetite. In a study conducted in rats, an 80% decrease in ghrelin hormone level was observed when the auxintic mucosa, where ghrelin hormone is released, was removed (Aroda et al. 2012). In this research, we found a reduction in ghrelin hormone levels in the GP and SG groups. However, ghrelin hormone levels similarly decreased when LG was administered. In addition, in the LG group, this hormone level was very close to the control.

When Brinkman evaluated the gastric tissues of the SG and GP treated groups histopathologically in his study, he observed that the fibrosis level of the gastric tissue in these groups was higher compared to the control group. In the comparison between SG and GP groups, it was reported that the fibrosis level of the SG group was higher than that of the GP group (Brinkman et al. 2017). In our research, when the gastric tissues of the SG and GP groups were examined under light microscopy, an increase in fibrosis levels was observed compared to the control and other study groups.

Bradnova et al. examined the metabolic changes occurring in the body after GP and observed that hemoglobin, insulin, glucose and ghrelin levels were close to normal (Bradnova et al. 2016). Similarly, in our study on rats, we observed improvements in ghrelin and blood glucose levels after GP. In this research, weshowed a significant decrease of 29.5% in the BMI indices of rats that underwent SG. We also observed that the ghrelin and blood glucose hormone levels of the LG group were even lower than those of the SG and GP groups.

Conclusion

In our research, we showed that LG is as effective as surgical methods applied for this purpose in eliminating obesity and normalizing hormone levels. These data have revealed that non-surgical methods can be applied in the treatment of obesity, a serious problem that is frequently seen in our time.

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