ORIGINAL RESEARCH

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Effects of "Longitudinal Gastrojejunostomy" on Malabsorption in a Rat Model of Short Bowel Syndrome

Kısa Bağırsak Oluşturulan Ratlarda "Longitudinal Gastrojejunostomi" Ameliyatının Malabsorbsiyon Üzerine Olan Etkilerinin Karşılaştırılması

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Abstract

Objective: Short bowel syndrome (SBS) is a serious and chronic disorder of inadequate absorption of nutrients and fluid from the intestines. The most significant symptom of SBS is malnutrition. Weight loss due to malnutrition raised the morbidity and mortality rates of patients to a disappointing level. The use of making longitudinal gastrojejunostomy is a technique to grow neomucosa or intestinal metaplasia in the gastric mucosal surface and increase the intestinal transit time.

Method: Three groups (Sham, SBS, longitudinal gastrojejunostomy) were created for 24 Wistar-Hannover rats. The 1st group was determined as the Sham group. SBS was induced in animals in group 2 with appropriate intestinal resection. Longitudinal gastrojejunostomy was additionally performed in rats with SBS in group 3. At the end of the 14th day all rats were weighted, euthanized, and blood sample was collected and anastomotic parts were resected for histopathological examination.

Results: Weight loss was significantly less in group 3 and vitamin B12 and GLP-2 levels were significantly higher in group 3 compared to group 2. Longitudinal antiperistaltic gastrojejunostomy promotes intestinal metaplasia in the gastric mucosa and increased the absorption surface.

Conclusion: This surgical procedure can be used to increase the absorption surface area in SBS. However, this needs to be supported by sufficiently robust clinical trials.

Keywords: Intestinal metaplasia, longitudinal gastrojejunostomy, short bowel syndrome

Öz

Amaç: Kısa bağırsak sendromu (KBS), bağırsaklardan besin ve sıvının yetersiz emiliminin şiddetli, kronik bir durumudur. Longitudinal gastrojejunostomi, mide mukoza yüzeyinde yeni bağırsak mukozası veya intestinal metaplazisi oluşturmak ve bağırsak geçiş süresini artırmak için düşünülmüş bir tekniktir.

Yöntem: Yirmi dört adet Wistar-Hannover rat 3 gruba ayrıldı. Birinci grup Sham grubu olarak belirlendi, 2. gruptaki hayvanlarda uygun bağırsak rezeksiyonu ile birlikte KBS oluşturuldu, 3. grupta KBS oluşturulan ratlara longitudinal gastrojejunostomi uygulandı. On dört gün sonra tüm sıçanlar tartıldı, ötenazi yapıldı ve kan örnekleri alındı. Histopatolojik inceleme için anastomoz kısımları rezeke edildi.

Bulgular: Grup 3'te kilo kaybı anlamlı olarak daha azdır ve grup 3'te grup 2'ye kıyasla vitamin B12 ve GLP-2 seviyeleri anlamlı olarak daha yüksektir. Longitudinal antiperistaltik gastrojejunostomi mide mukozasında intestinal metaplaziyi teşvik ettiği ve emilim yüzeyini artırdığı gözlenmiştir.

Sonuç: Bu cerrahi işlem kısa bağırsak sendromunda absorpsiyon yüzey alanını artırmak için kullanılabilir. Ancak bunun yeterince güçlü klinik deneylerle belirlenmesi gerekmektedir.

Anahtar kelimeler: İntestinal metaplazi, kısa bağırsak sendromu, longitudinal gastrojejunostomy

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Introduction

Short bowel syndrome (SBS) is a severe, life threatening condition of inadequate absorption of aliments and liquids from the intestines, which can be acquired or congenital. It is characterized by a reduction in the length of the intestines. After the procedure of intestinal resection, SBS is one of the major causes leading to intestinal failure (IF) (1,2). Small bowel atresia, volvulus, necrotizing enterocolitis, and invagination are the main clinical conditions of SBS in children. Strangulated bowel resection, inflammatory bowel diseases, trauma and ischemia are the most common causes leading to SBS in adults.

Important symptoms in these patients are; malnutrition, dehydration, diarrhea and steatorrhea. The severity of these symptoms are correlated to the length of the remnant small bowel (3). The leading clinical symptoms of SBS are malnutrition and loss of weight. A variety of clinical and surgical procedures, as well as parenteral nutrition (PN), have been used in the treatment of patients with SBS and in animal experiments. However, only minimal clinical progress could be acquired so far. Despite all the developments and studies, the morbidity and mortality rates in patients with SBS are disappointingly high. Patients with SBS are somewhat difficult to follow-up and patient survival is closely related to the adaptive capacity of the remaining intestines. However, proper absorption of the intestines may be possible with surgical treatments consisting of reconstructive procedures of the remaining intestine and intestinal transplantation (4). The purpose of reconstructive procedures is to prolong the intestinal transit time and accelerate the development of new intestinal mucosa (neomucosa). All these studies are still experimental (5). These studies for non-transplant procedures for the treatment of SBS can be divided into four categories: (1) Maximizing the remaining segments of the bowel (closure of stomas, minimizing nutritional loss due to enterocutaneous fistulas, and removing obstructions and blind loops) (2) improving peristalsis of dilated and/or dysmotile bowel by tapering or inversion plication (3) delaying the rapid passage of intestinal contents by reversing intestinal segments, forming semi-obstructive valves, or interpositioning the colon between a divided proximal jejunum; and (4) autologous reconstruction with intestinal lengthening, i.e., Longitudinal Intestinal Lengthening and Tailoring (LILT) (Bianchi) and Serial Transverse Enteroplasty (STEP), or sequential combinations of the two.

Published studies have been demonstrated the function of absorption in intestinal metaplasia of the stomach (6). The use of making longitudinal gastrojejunostomy is an intestinal mucosal/intestinal metaplasia augmentation technique in gastric mucosal surface and increase the intestinal transit time. Growing neomucosa/intestinal metaplasia is used for enlargement of absorbtive surface of the intestines.

In our experimental study we aim to investigate the effects of antiperistaltic, longitudinal gastrojejunostomy on malnutrition and weight loss.

Materials and Methods

Experimental design: Twenty-four male Wistar-Hannover rats (350-450 g), obtained from University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital Animal Center (BADABEM), were fed with standard pellet, ad libitum. Ethic approval gained from the University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital Animal Care and Use Committee (2015-39).

Animal groups and surgical procedure: Rats were randomly and equally divided into 3 groups. Rats were anesthetized by an isoflurane (5% for induction and 2% for maintenance, Isoflurane® Baxter, Puerto Rico, USA). Each rat was weighed and recorded preoperatively. 3 cm midline abdominal incision was done. In group 1 (sham) stomach and jejunum were manipulated and revealed. Incisions closed without intervention. In groups 2 and group 3; the jejunum and ileum segments between 10 cm proximal to the ileocecal area and 10 cm distal from the Treitz ligament were resected, and anastomosis was performed with 7/0 polyproplen (Doğsan®, Trabzon, Turkey) suture. Additionally, in group 3; 50% of glandular part of the stomach was separated in longitudinal axis with 3/0 silk suture (Doğsan®, Trabzon, Turkey). The separated stomach was opened with a 1 cm incision in the longitudinal plane at the level of the large curvature and a 1 cm jejunotomy performed to the proximal jejunum and longitudinal, anti-peristaltic anastomosis was performed between these two tissues. This anastomosis was made proximal of the entero-enteral anastomosis (Figure 1). After these procedures, the abdomen was closed anatomically. After 14 days, all subjects were euthanized under anesthesia and blood was drawn by cardiac puncture and centrifuged for the measurement of biochemical parameters. (ALT, AST, magnesium, calcium, iron, glucagon like peptide-2, albumin and vitamin B12). En bloc resection of gastrojejunostomy was performed and 10% formaldehyde solution is used for tissue fixation for histopathological examination.

Histological analysis: The anastomotic parts were processed for paraffin embedding. Four micron paraffin sections were obtained and stained with hematoxylin-eosin. The tissue blocks that have minimum artifacts were gathered for immunohistochemical evaluation with MUC 1 (CellMarque 290M-14) and MUC2 (CellMarque 291-14) anticores. Alcian Blue (pH: 2.5) staining was performed for the intestinal type mucin examination.

Biochemical analysis: Blood samples were centrifuged for 10 minutes in 4000 rpm at +4 °C. Serum samples were stored at -80 °C. Glucagon like peptide-2 assay was carried out using "Micro Elisa" method. Sunred® Biological Technology Co. (Shangai, China) Micro Elisa kits were used in this process. All micro elisa measurements were performed on DAR800 micro elisa reader at 450 nm wavelength.

Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program was used for the statistical analysis. Descriptive statistical methods (standard deviation, mean), Mann-Whitney U test (group comparison) and chi-square test (comparison of qualitative data) were used. A p-value of <0.05 was considered statistically significant.

Results

No differences were observed between groups in food intake and no complications were observed in animals.

Body weight monitoring: Rats were weighed and recorded separately at the beginning and end of the surgical procedure. The mean weight losses of the groups (1-3) at the end of the experiment were determined as +2.79 gr, -38.88 gr and -34.61 gr, respectively. Weight loss was significantly low in group 3 compared to group 2 ($p<0.05$) (Table 1).

Histological evaluation: Intestinal metaplasia was determined in all rats of group 3 (Figure 1). Stratified epithelium was observed in the gastric mucosa in one specimen in group 3 (Figure 2).

Biochemical evaluation: No significant differences observed between group 1, 2 and 3 in terms of albumin, ALT, AST, Ca, Iron and Mg levels, but there was statistically difference between groups 2 and 3 in terms of vitamin B12 and GLP-2 levels (Table 2).

Discussion

Inability of the gastrointestinal tract to maintain sufficient and proper absorption and digestion without PN defined as IF. The clinical management of SBS is quite difficult and complicated. In cases which PN is not sufficient, a multidisciplinary approach that also requires surgical intervention should be used. Unfortunately, despite advances, these complicated and difficult treatment options are still associated with high morbidity and mortality rates (7,8). In addition to the benefits of PN, especially in its prolonged clinical use; it brings many major complications such as resistant hospital infections, thromboembolism, catheter problems, metabolic complications (renal or liver diseases) and ultimately organ failure (9). Several surgical procedures have been described for the management of SBS, such as construction of reversed intestinal segments, interposition of colon, and lengthening procedures. However, a clear gold standard procedure has not yet to be determined. Intestinal transplantation and autologous

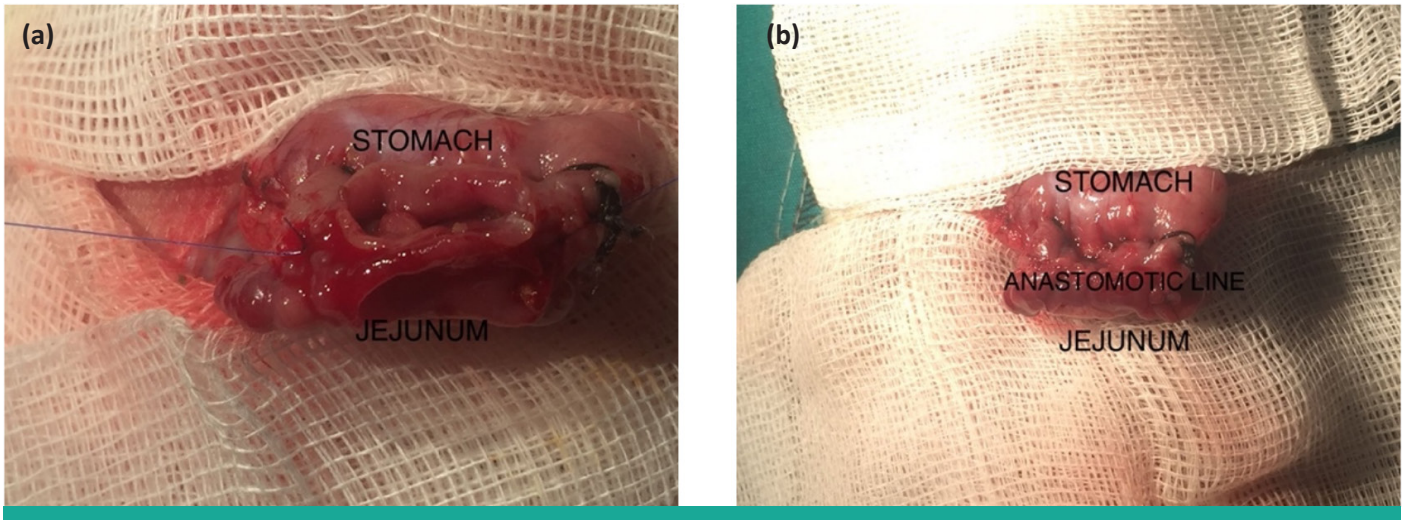

Figure 1. Surgical procedure. (a) and (b) Anastomotic line is shown between the separated gastric partition and ileum

reconstruction procedures consisting of an enlarged mucosal surface area and elongated bowel are the most recommended surgical interventions (7). Accepted autologous intestinal reconstruction procedures for the elimination of PN dependence are Bianchi procedure (LILT) and the STEP. These surgical approaches have major complications such as leakage of the stapler line, bleeding and stricture of the intestinal lumen. Intestinal transplantation is a very complex surgical intervention with high morbidity and mortality rates all over the world. It should be performed by experienced surgeons in specialized centers. For these reasons, it should be considered as the last treatment option (10,11).

Intestinal metaplasia of the stomach has an absorptive function which has been described previously in the literature (6). Growing intestinal metaplasia in gastric mucosa is a technique for expanding the absorptive surface volume of the intestinal mucosa. The use of gastric mucosa to grow intestinal metaplasia in SBS has not been described before and this study is the first experimental model in the literature as we know.

Weight loss is a significant clinical condition in patients with SBS and therefore weight monitoring is an important elimination of PN dependence are Bianci procedure follow-up criteria and it is a frequently used method in SBS studies. Weight gain or reduction in weight loss are a major complications such as a leakage of the stapler line, good indicator for progression in patients with SBS. Studies have reported various percentages of weight loss. Swartz-Basile et al. (12) reported a 10-20% weight loss in their study, Washizawa et al. (13) established a 10.8% weight loss, Koruda et al. (14) reported a 5.7% weight loss over a 1 week follow-up period. In the present study, we observed a 17.56% (38.88 gr) and 15.67% (34.61 gr) (group 2 and 3 respectively) over a 14 days follow-up period. A statistically significant difference was found between group 2 and 3 ($p<0.05$).

Electrolyte disturbances are important reason for morbidity in patients with SBS due to insufficient absorption.

Table 1. Body weight monitoring and the differences before and after the procedure

	Group 1	Group 2	Group 3
Before the procedure	217.81±21.1	221.33±25	220.75±24.5
After the procedure	220.6±24.1	182.45±11	186.14±11.7
Difference	2.79	-38.88*	-34.61*

*($p<0.05$)

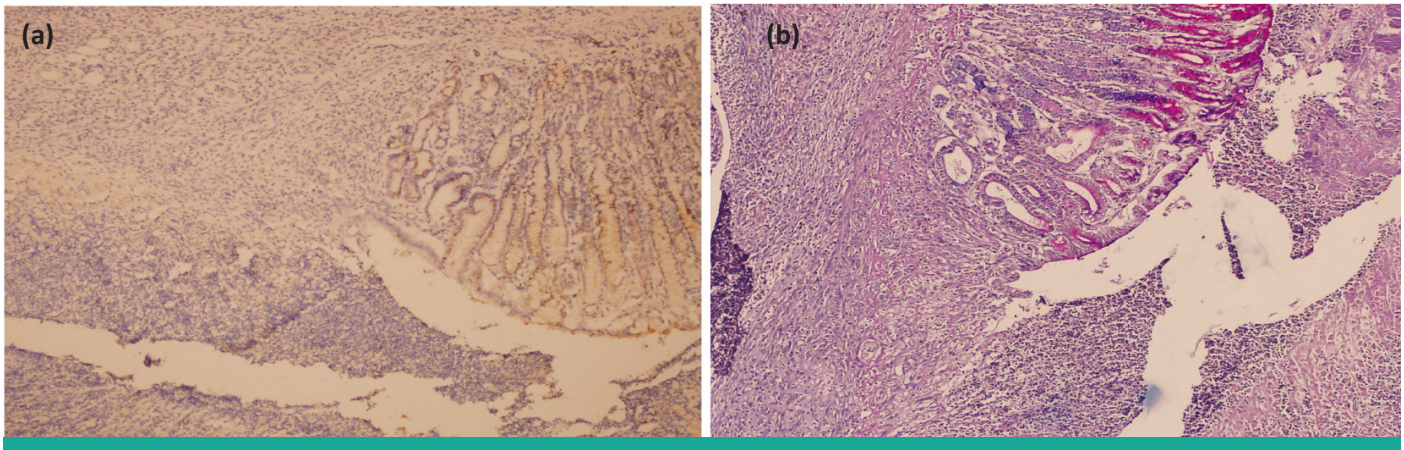

Figure 2. Intestinal metaplasia in the anastomotic line. (a): Hematoxylin and eosin (HE) x220, (b): Alcian blue (AB) x220

Table 2. Mean and standard deviations of biochemical values of the groups

	Albumin (g/dL)	ALT (U/L)	AST (U/L)	Ca (mg/dL)	Iron (mg/dL)	Mg (mg/dL)	Vit B12 (pg/mL)	GLP-2 (ng/mL)
Group 1	4.3±0.72	54.6±4.59	101.6±19.2	9.8±0.37	196.6±89	2.77±0.5	464±80.9	2.012±0.54
Group 2	3.22±0.71	41.51±5.42	118.43±41.9	9.695±0.84	108.31±41	2.52±0.2	374.83±82*	0.412±0.11*
Group 3	3.71±0.5	42.01±4.9	127.22±35.1	9.668±0.73	148.16±119	3.24±0.8	787.01±511*	1.845±1.08*

*($p<0.05$), ALT: Aspartate aminotransferase, AST: Alanine aminotransferase, CA: Calcium, GLP-2: Glucagon-like peptide-2, Mg: Magnesium

Albumin, iron, magnesium, vitamin B12, calcium, AST and ALT levels are the significant indicators of nutritional status and liver function of the patient (15-18). Biochemical evaluation revealed that there were no statistically significant differences between the groups in levels of albumin, AST, ALT, Ca, iron and Mg, but group 3 had higher levels of vitamin B12 which is statistically significant ($p<0.05$).

Intestinal L-cells secrete Glucagon-like peptide-2 (GLP-2) after food intake. GLP-2 has effects on the intestines such as stimulating growth, increasing absorption, promoting regeneration and regulating integrity of the intestinal epithelium (19). Drucker et al. (20) reported that, GLP-2 was found to only stimulate the development of colon and small intestine tissues in mice, but had no effect on other gastrointestinal tissues. In SBS, GLP-2 performs its intestinal absorption-enhancing effect by increasing the development of intestinal tissues and slowing gastric emptying and gastro-intestinal transit time (20,21). In present study GLP-2 values of group 3 were significantly higher than group 2 ($p<0.05$).

Conclusion

In the evaluations 2 weeks after the formation of SBS in rat; it has been observed that "antiperistaltic, longitudinal gastrojejunostomy" has a reducing effect on weight loss due to massive resection of small bowel and promotes serum levels of vitamin B12 and GLP-2 which are essentials in the treatment of the patients with SBS. This surgical procedure may be beneficial in the treatment of SBS for expanding the total surface of intestinal absorption. Nevertheless, despite the positive results, this study was limited by the small amount of rats and short experimental follow-up period. Due to the limited number of rats used in the experiment and the short follow-up period, our study needs to be developed with other studies. More studies are needed before firm conclusions can be drawn about the safety and advantages of this surgical procedure. In the potential use of this surgical technique in patients with SBS, some possible side effects such as gastric ulcer or malignancy due to intestinal metaplasia should also be taken into account. Therefore, this experimental study has been an incentive for further studies.

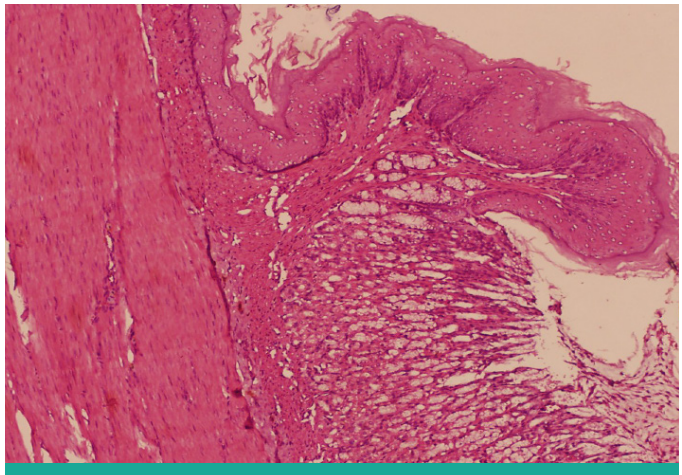

Figure 3. Stratified epithelium in the gastric mucosa (x220)

Ethics

Ethics Committee Approval: All experimental procedures were approved by the University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital Animal Care and Use Committee (2015-39).

Informed Consent: It is not necessary as it is an animal test.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.B., A.K.A., C.E., Concept: S.A., O.B.G., A.Ç., Design: S.A., O.B.G., A.Ç., Data Collection or Processing: S.A., A.B., A.K.A., C.E., Analysis or Interpretation: O.B.G., A.Ç., H.Y., A.K.A., Literature Search: C.E., A.Ç., H.Y., A.B., Drafting Manuscript: S.A., A.Ç., E.Y., H.Y., Critical Revision of Manuscript: C.E., A.Ç., H.Y., A.B., Supervision: A.Ç., O.B.G., E.Y., Writing: S.A., O.B.Ç., A.Ç., E.Y.

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