Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2023;48(4):1365-1373
DOI: 10.17826/cumj.1370487

RESEARCH

Effects of sleeve gastrectomy on N6-methyladenosine modification in duodenal tissue

Duodenal dokuda N6-metiladenozin modifikasyonu üzerine sleeve gastrektomi etkileri

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Abstract

Purpose. In this study, the expression levels of METTL3, METTL14; and WTAP genes involved in the N6-methyladenosine (m6A) pathway, regulating cellular processes including cell renewal, differentiation, invasion; and apoptosis, were investigated in duodenal tissue after sleeve gastrectomy (SG).

Material and Methods: Duodenal biopsies were obtained from 24 obese patients before (BG) and 6 months after (AG) sleeve gastrectomy. Pathological evaluation was performed by H&E staining. Expression levels of METTL14, METTL3, and WTAP genes were analysed by Real-Time PCR.

Results: The METTL14 gene was significantly increased after surgery (BG-AG Median (Q1-Q3): 0.36 [0.07-1.52]-9.83 [0.20-68.12]). METTL3 (BG-AG Median (Q1-Q3): 0.49 [0.32-0.84]-0.67 [0.36-20.5]) and WTAP (BG-AG Median (Q1-Q3): 0.95 [0.17-7.65]-5.54 [2.34-66.07]) gene expressions were not altered. However, a strong positive (r=0.692) and significant relationship was found between METTL3 and WTAP. Chronic inflammation 81.32 %, intestinal metaplasia 10.54 %, activity 68.88 %, atrophy 5.32 %. No malignant findings were detected.

Conclusion: For the first time, we demonstrated that sleeve gastrectomy may increase the expression of the METTL14 gene involved in the m6A-methyladenosine pathway in duodenum. This alteration may lead to gastrointestinal tract diseases.

Keywords:. Sleeve gastrectomy, gene expression, N6-methyladenosine, METTL14, obese patients.

Öz

Amaç: Bu çalışmada hücre yenilenmesi, farklılaşma, invazyon ve apoptozu içeren hücresel süreçleri düzenleyen N6-metiladenozin (m6A) yolağında görevli METTL3, METTL14 ve WTAP genlerin ifade düzeyleri sleeve gastrektomi (SG) sonrasında duodenum dokusunda araştırılmıştır.

Gereç ve Yöntem: 24 obez hastadan SG öncesinde (BG) ve 6 ay sonra (AG) duodenum biyopsileri alındı. H&E boyama ile patolojik inceleme yapıldı. METTL3, METTL14 VE WTAP genlerinin ifade düzeyleri Real-Time PCR ile analiz edildi.

Bulgular: METTL14 geninin ifade düzeyinin cerrahi sonrasında anlamlı derecede yükseldiği tespit edildi (BG-AG Medyan (Q1-Q3): 0.36 [0.07-1.52]-9.83 [0.20-68.12]). METTL3 (BG-AG Medyan (Q1-Q3): 0.49 [0.32-0.84]-0.67 [0.36-20.5]) and WTAP (BG-AG Medyan (Q1-Q3): 0.95 [0.17-7.65]-5.54 [2.34-66.07]) genlerinin ifade düzeylerinin değişmediği belirlendi. Ancak, METTL3 ve WTAP genleri arasında kuvvetli pozitif (r=0.692) ve anlamlı ilişki olduğu belirlendi. %81.32 kronik inflamasyon, %10.54 intestinal neoplazi, %68.88 aktivite, %5.32 atropi olduğu belirlendi. Malignansi bulguları saptanmadı.

Sonuç: Bu çalışma ile ilk defa sleeve gastrektominin duodenum dokusunda, m6A-metiladenozin yolağında görevli olan METTL14 geninin ifadesini artırdığı gösterilmiştir. Bu değişim gastrointestinal yolak hastalıklarına yol açabilir.

Anahtar kelimeler: Sleeve gastrektomi, gen ekspresyonu, N6-metiladenozin, METTL14, obez hastalar.

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Received: 03.10.2023 Accepted: 15.12.2023

INTRODUCTION

Currently, bariatric surgery is considered the most effective approach for addressing morbid obesity. Sleeve gastrectomy, in particular, is becoming increasingly popular as a preferred intervention for individuals with obesity[1]. This procedure is also used in the treatment of type 2 diabetes[1,2]. Sleeve gastrectomy involves the removal of 80% of the stomach tissue, resulting in a reduction in stomach volume and food intake. However, it also alters the amount of hormones and enzymes secreted by the stomach. Studies have shown that DNA methylation and expression of non-coding RNAs change after bariatric surgery. Bariatric surgery may be able to reprogram or reverse the epigenetic profile associated with obesity. These epigenetic regulations appear to have a positive impact on metabolic disorders such as insulin resistance, hypertension, and cardiovascular disease[3].

Epigenetic changes refer to modifications in DNA methylation, chromatin and histone structures, and noncoding RNA. These reversible changes regulate gene expression in response to various cellular conditions. It is suggested that the SG process may cause epigenetic rearrangements in the expression of many genes associated with inflammation, insulin resistance, and lipid metabolism[4-6]. However, no studies have yet investigated the epigenetic changes in duodenal tissue after surgery. Recent studies have shown that post-transcriptional epigenetic modifications play a crucial role in many physiological processes and disease development. One such modification is the methylation of the N6 position of adenosine, known as N6-methyladenosine (m6A), which occurs in eukaryotic mRNA. This modification regulates the expression of genes involved in cellular processes, including cell differentiation, regeneration, apoptosis, and invasion[7]. The m6A modification regulates the eukaryotic transcriptome by controlling mRNA splicing, localization, translation, and stability. The methylation of m6A is carried out by m6A methyltransferases known as writers, removed by demethylases (erasers), and recognized by reader proteins that regulate RNA metabolism. These processes determine the future of the modified transcriptome[8].

Alterations in m6A methylation profile contribute to cancer pathogenesis and development by causing changes in the expression of tumor-related genes such as BRD4, MYC, SOCS2 and EGFR[9,10]. The methyltransferase complex, consisting of the catalytic subunit METTL3 and accessory subunits METTL14, WTAP, VIRMA, RBM15, and ZC3H13, sets up m6A simultaneously with transcription. METTL14 forms a stable complex with METTL3 and plays a key role in substrate recognition. Wilms tumour 1 associated protein (WTAP) localises the METTL3-METTL14 heterodimer and initiates its catalytic activity[11]. Recent research has shown that m6A-methyladenosine modifications play an active role in cancer through various mechanisms. However, studies on gastrointestinal tract cancers are limited[12-17].

The aim of this study is to investigate the epigenetic changes that occur in the duodenal tissue following sleeve gastrectomy (SG), a procedure conducted to reduce stomach volume. Sleeve gastrectomy is considered an effective method for treating metabolic issues such as obesity and type 2 diabetes. The SG procedure involves removing 80% of the stomach tissue, which reduces both stomach volume and food intake, while also altering the secretion of hormones and enzymes by the stomach. The research investigates whether bariatric surgery can reverse the epigenetic profile associated with obesity by examining the epigenetic regulations of this procedure.

The study focuses on the epigenetic changes induced by bariatric surgery in the duodenal tissue, addressing a knowledge gap. Previous research has mainly examined epigenetic alterations in stomach tissue following bariatric surgery, with limited investigation into the duodenal tissue.

The study investigates the impact of hormonal changes following sleeve gastrectomy on the RNA methylation profile. The reduction in stomach volume and hormonal regulations may both influence the epigenetic profile. To shed light on these aspects, the study aims to investigate the expression levels of key genes involved in the m6A methylation pathway, such as METTL3, METTL14, and WTAP, in the duodenal tissue after sleeve gastrectomy. These genes are involved in regulating m6A methylation and are believed to significantly impact the expression of writer genes. Therefore, permanent alteration of the transcriptome can potentially lead to various pathological changes in tissues, particularly cancer. The study emphasizes the effects of m6A

methylation on cancer pathogenesis and development, seeking to determine the potential role of these epigenetic changes in the gastrointestinal system of patients undergoing tube stomach surgery due to obesity.

MATERIALS AND METHODS

Patients and tissue preparation

Ethics committee approval was received from Mersin University Clinical Research Ethics Committee with the decision number 2022/213 dated 06.04.2022. In this study, informed consent was obtained from all individual participants. Respondents were assured that their original data would be kept confidential and not shared. The study was conducted in accordance with the Principles of the Declaration of Helsinki. Duodenal tissue biopsy samples were collected by experienced general surgery from patients at the General Surgery Clinic of Bakirkoy Dr. Sadi Konuk Training & Research Hospital using an endoscopic forceps. RNA isolation and real-time PCR analyses were conducted by medical biology and genetics experts at the Atlas Biotechnology laboratory. Pathology analyses were performed by experienced pathologists at Bakirkoy Dr. Sadi Konuk Training & Research Hospital.

This study included 24 patients with obesity who underwent SG for morbid obesity. Patients were eligible if they had a BMI (body mass index) greater than 40 kg/m² or a BMI greater than 35 kg/m² with associated comorbidities, such as diabetes mellitus, hypertension, obstructive sleep apnea, or orthopedic problems.

The study included patients who met the following criteria: previous unsuccessful attempts with non-surgical weight loss methods, age between 18-65, absence of oral intake disorders, absence of malignant findings, and having undergone sleeve gastrectomy procedure between January 1, 2021, and February 28, 2021, and who signed the informed consent form (ICF). The surgical group excluded patients with eating disorders, those with obesity caused by adrenal masses, individuals under 18 or over 65 years of age, those with oral intake disorders, and patients with evidence of malignancy. The study also excluded individuals who did not sign the informed consent form (ICF).

Duodenal biopsies were taken before and 6 months after surgery for pathological and genetic analyses. One of the biopsy samples was placed in RNA stabilisation reagent (Qiagen) and stored at -80°C until RNA isolation. The other sample was stored in 10% buffered formalin fixative in sterile bottles for pathological examination.

Pathological evaluation of tissues stained with hematoxylin-eosin

Biopsies were analyzed by routine techniques using hematoxylin and eosin. It was also stained with Giemsa to identify Helicobacter pylori organisms. Slides were assessed by two pathologies, and each morphological variable was scored from 0 to +3 (nil, mild, moderate, marked). Lymphocytes and plasma cells were recorded. Neutrophil and H. pylori density were determined. Intestinal metaplasia and glandular atrophy were noted and then classified into three categories. Tissues stained with H&E were evaluated pathologically in terms of intestinal metaplasia, chronic inflammation and atrophy.

RNA extraction

Total RNA was isolated from tissues using the RiboEx (GeneAll, Cat:301-001) RNA isolation kit. A Nanodrop spectrophotometer was used to measure RNA concentration. The cDNA synthesis was performed using the HyperScriptTM first-strand synthesis kit (GeneAll, Cat: 601-005). The qRT-PCR reaction was performed according to the kit protocol. The Real-Time qPCR reaction was performed using RealAmpTM SYBR qPCR Master mix (GeneAll, Cat: 801-051). Real-Time qPCR reaction was performed using the Applied Biosystems ViA 7™ Real-Time PCR under the kit protocol.

Detection of METTL3, METTL14, and WTAP Expression with ΔΔCT Method of qRT-PCR

Beta-actin (Actb) gene expression was examined as an endogenous control. The expression levels of METTL3, METTL14, and WTAP genes were determined by real-time PCR. The studies were performed on ice. When quantifying mRNA expressions, the Actb transcript was used as a reference and normalized relative to the control group. The "ΔΔCt method" (also known as $2^{-\Delta\Delta Ct}$ method) was used to calculate the relative quantification. Ct values over 35 cycles were considered undetectable. Actb was used as an endogenous control was accepted as 1.0.

Statistical analysis

The sample size calculation for the study was conducted using the statistical package program G*Power 3.1.9.4. Due to the absence of a similar study in the literature search scope for comparison, effect size was set at 0.50, based on the results determined by Jacob Cohen[18]. To assess sample size, power calculations were carried out considering a desired study power of 80% and an α error of 0.05, one-tailed. The minimum sample size was determined as 24 patients. The assumptions of normality of the data were tested by Shapiro Wilk test.

Descriptive statistics of data without normal distribution were given in median and quarters (Q1-Q3). Expression data for the METTL3, METTL14, and WTAP genes before and after gastrectomy were assessed using the Related-Samples Wilcoxon test, as the data did not exhibit a normal distribution. The relationship between gene expressions (METTL3, METTL14, and WTAP genes) was analyzed using Spearman's rho correlation analysis, as the gene expressions did not exhibit a normal distribution. The pathology data (chronic inflammation, intestinal metaplasia, activity, atrophy) were determined as percentages using descriptive statistical. All statistical analyses were performed using the SPSS software 21 and the MedCalc Free Trial statistical software package. The P<0.05 value was defined as being statistically significant.

RESULTS

Of the patients, 74.4 % were female, and 25.6 % were male. Mean age was 38.5 – 10.6 years (18–64), mean weight was 130.8 kg (94–240 kg), and mean body mass index was 47.4 kg/m^2 (36–106 kg/m^2). The results of parameters in pathologic materials of biopsy are as follows: Chronic inflammation 81.32 %, intestinal metaplasia 10.54 %, activity 68.88 %, atrophy 5.32 %. No malignant findings were detected in any of the patients in the study group.The expression of the studied genes in the tissues was also increased 6 months postoperatively compared to preoperatively. Before gastrectomy (BG) METTL3 gene expression median value was 0.49, after gastrectomy (AG) it was 0.67. Also, WTAP gene expression median value was 0.95 before and 5.54 after gastrectomy. It was determined that surgery not altered METTL3 (P=0.203) and WTAP (P=0.074) expression levels. However METTL14 gene expression level significantly altered after gastrectomy (P<0.05) (Figure 1). The median value of METTL14 expression level was 0.36 before gastrectomy and 9.83 after gastrectomy (Table 1).

Table 1. Expression values of METTL3, METTL14, and WTAP genes in duodenal tissues before (BG) and 6 months after (AG) surgery.

Genes		Expression Value ($2^{-\Delta\Delta CT}$) Median	25%	75%	P
METTL3	BG	0.49	0.32	0.84	0.203
	AG	0.67	0.36	20.5	
METTL14	BG	0.36	0.07	1.52	0.028
	AG	9.83	0.20	68.12	
WTAP	BG	0.95	0.17	7.65	0.074
	AG	5.54	2.34	66.07	

When compared with Actb (housekeeping gene- beta-actin), it was determined that METTL14 expression level significantly increased 6 months after the surgery in duodenum tissue (P<0.05). But there were no alterations in METTL3 and WTAP genes expression levels after the surgery (P>0.05).

Also we determined correlations between the genes. There was a strong positive (r=0.692) and significant (p≤0.01) relationship was found between METTL3 and WTAP (Table 2). METTL3 and WTAP gene expressions increase significantly and with a strong relationship. The variance explained by the variables on each other is 47.88%. In other words, 47.88% of the increase in WTAP gene expression may be caused by the METTL3 gene. This relationship is shown in Figure 2.

Table 2. The relationship between the expressions of METTL3, METTL14 and WTAP genes

Correlations		METTL3	METTL14	WTAP
METTL3	Spearman's rho	1.000	0.262	0.692**
	p	.	0.265	0.001
	N	24	24	24
METTL14	Spearman's rho	0.262	1.000	0.386
	p	0.265	.	0.092
	N	24	24	24
WTAP	Spearman's rho	0.692**	0.386	1.000
	p	0.001	0.092	.
	N	24	24	24

** Strong positive (r=0.692) and significant (p≤0.01) relationship was found between METTL3 and WTAP genes expressions. 47.88% of the increase in WTAP gene expression may be due to the METTL3 gene. **. Correlation is significant at the p≤0.01 level (2-tailed).

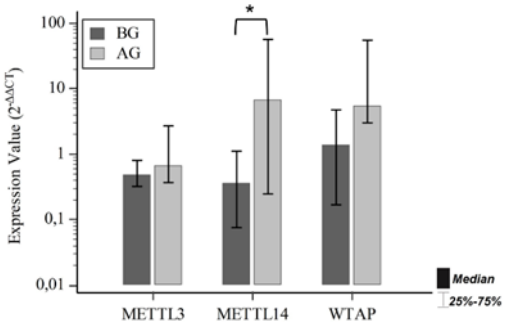

Figure 1. The expression levels of METTL3, METTL14, and WTAP were compared with the control group 6 months after surgery.

Biopsy material taken before sleeve gastrectomy (BG) was accepted as a control group. The expression levels of METTL3, METTL14, and WTAP were compared with the control group 6 months after surgery (AG). A statistically significant increase was shown for METTL14 (p≤0.05).

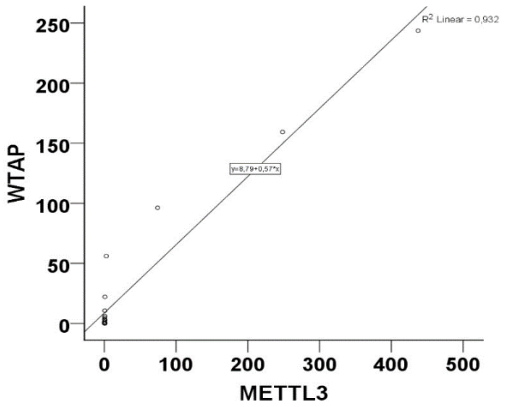

Figure 2. Graphical representation of the correlation between the expressions of METTL3 and WTAP genes.

METTL3 and WTAP gene expressions increase significantly and with a strong relationship. Strong positive (r=0.692) and significant (p≤0.01) relationship was found between METTL3 and WTAP genes expressions.

DISCUSSION

Sleeve gastrectomy (SG) is the most common surgical treatment for morbid obesity. This surgery causes permanent changes in the level of hormones and enzymes secreted by the gastric tissue. Furthermore, weight loss due to systemic and metabolic changes causes molecular-level alterations in many parameters. However, it remains unclear whether this widely used procedure causes damage to the gastrointestinal system, including cancer, over the patient's lifetime. There is a growing body of research indicating that surgical treatment can lead to changes in epigenetic modifications[4-6]. One such modification is the m6A methyladenosine process, which plays a crucial role in determining the fate of the RNA epitranscriptome. It is worth noting that m6A regulators are predominantly upregulated in gastrointestinal cancers. Additionally, recent studies have demonstrated a correlation between the expression levels of m6A regulators and the survival rates of cancer patients[15].

Numerous studies have been conducted on gastrointestinal tract diseases and epigenetic changes following SG. Epigenetic changes resulting from surgical treatment primarily focus on systemic effects,

including inflammation, insulin resistance, and lipid metabolism[19,20]. Although the gastrointestinal tract has been examined pathologically, no previous research has investigated epigenetic changes in this area. Thus, our aim was to investigate the expression levels of the METTL3, METTL14, and WTAP genes, which are responsible for m6A modification, in duodenal tissue following a six-month period of SG.

SG treatment regulates the secretion of various hormones and enzymes. One of the most significant hormones affected by surgery is ghrelin[21,22], which regulates gastrointestinal tract (GIS) motility and activates the enzyme nitric oxide synthase in the endothelium. A meta-analysis was conducted to investigate changes in gut hormones after SG. The study found that levels of hormones such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and gastric inhibitory peptide (GIP) were affected[21,22]. These postoperative changes may cause epigenetic rearrangements in patients. While these epigenetic changes may regulate the metabolic activities of patients, they may also cause malignant changes in the tissues of the gastrointestinal tract.

Controversial results exist regarding the relationship between bariatric surgery and cancer risk. Mackenzie et al. found a reduction in hormone-related cancers (breast, endometrial, and prostate) following bariatric surgery[23], while Aravani et al. reported no difference in colorectal cancer risk between surgical and nonsurgical groups[24]. A study found that 35% of patients who underwent SG had gastritis, 19.2% had esophagitis, 23.1% had hiatal hernia, and 2.6% had benign gastric polyp, based on 5-year postoperative endoscopy findings. Barrett's esophagus, which increases the risk of developing esophageal cancer, was observed in 1.3% of patients[25]. Our postoperative 6-month pathologic findings showed chronic inflammation in 81.32% of cases, intestinal metaplasia in 10.54%, activity in 68.88%, and atrophy in 5.32%. SG treatment may cause pathological changes in gastrointestinal tissues. However, there is currently insufficient evidence to link SG treatment with an increased risk of gastrointestinal cancer. These molecular changes may provide a basis for further research.

It is widely acknowledged that m6A-methyladenosine mRNA methylation profiles play a crucial role in cancer development and progression. It is important to note that these findings are still being investigated and further research is needed to fully understand the implications of these modifications in cancer. These modifications have been shown to rearrange the expression levels of genes involved in tumorigenesis and may also be associated with patient survival[14]. A study conducted on esophageal cancer revealed an increase in METTL3, WTAP, YTHDF1, and YTHDF2, which are involved in the m6A-methyladenosine pathway[12]. Xia et al. reported that the expression of METTL3 was increased in esophageal squamous cell carcinoma and could be used as a prognostic biomarker[13]. In vivo and in vitro studies in gastric cancer have shown that the expression of YTHDF2 is lower than that in normal tissues and cells. YTHDF2 inhibits the growth of gastric cancer cells, and its expression level is also associated with survival[15]. The m6A sequence and phospho-MAPK array analyses showed that the PI3K/Akt and mTOR signaling pathways are affected by m6A modifications. These modifications are involved in the development of gastrointestinal cancer through these signaling pathways[17].

This study examines the epigenetic changes following postoperative metabolic pathways in the gastrointestinal tract. Specifically, we investigate the alteration of M6A modifications after SG. Our examination includes a pathological and genetic analysis of duodenal tissue. The study revealed an upregulation of METTL3, METTL14, and WTAP genes in duodenal tissue. Notably, postoperative alterations were found to affect the expression levels of METTL14 gene. Pathological findings, including chronic inflammation, intestinal metaplasia, activity, and atrophy, were observed. No malignant indications were detected. Therefore, it can be concluded that surgery may cause epigenetic changes in the m6A-methyladenosine signaling pathway, which can affect the expression levels of METTL14. Recent evidence supports a significant increase in METTL14 expression. Studies on gastric cancer (GC) and colorectal cancer (CRC) have revealed that METTL14-mediated m6A modification plays a crucial role in controlling the progression of CRC and GC. Experimental studies and bioinformatics have confirmed that METTL14 is highly expressed in colorectal cancer (CRC) and gastric cancer (GC) compared to normal tissues. High levels of METTL14 expression are closely associated with a better prognosis for patients. These results suggest that the METTL14 gene may inhibit the metastasis and proliferation of CRC and GC through various pathways and mechanisms[26,27]. For instance, Chen et al. found that overexpression of METTL14

significantly increased the m6A levels of CRC cells and inhibited in vitro CRC proliferation and metastasis, while loss of METTL14 had the opposite effect[28]. They demonstrated that METTL14 and YTHDF2 work together to regulate m6A methylation modification and inhibit the migration, invasion, and metastasis of CRC both in vitro and in vivo[29].

In their study, Wang et al. discovered that METTL14 could increase the expression of the tumor suppressor protein Kruppel-like factor 4 (KLF4), which inhibited the invasion and metastasis of CRC cells[30]. Downregulation of METTL14 in GC and CRC has been found to suppress the aggressive phenotype of GC by inactivating the PI3K/AKT/mTOR signaling axis[29,31]. Additionally, Zhang et al. discovered that METTL14 deficiency activates the Wnt and PI3K-Akt signaling pathways, triggering the proliferation and invasion of GC cells in vitro. They also identified a potential correlation between m6A levels, immunotherapy features, and interferon signaling in METTL14-deficient cells[32]. The study showed that METTL14 is an independent determinant of survival and inhibits metastasis in both CRC and GC both in vivo and in vitro[33,34].

The recent research findings support the significance of increased METTL14 expression levels as a prognostic factor. Furthermore, our study found a strong correlation between the METTL3 and WTAP genes, which is consistent with Sorci et al.'s findings. They reported that METTL3 levels are critical for maintaining WTAP protein homeostasis and that overexpression of the METTL3 protein leads to upregulation of the WTAP protein. The study demonstrated that METTL3 levels can regulate WTAP expression through direct and indirect mechanisms that affect mRNA stability and translation[35]. Therefore, we concluded that an increase in METTL3 expression leads to an increase in WTAP gene expression. This study is the first to investigate m6A writer expression levels in the duodenum after sleeve gastrectomy. Our study found that surgery can increase the expression of the METTL14 gene, which is responsible for m6A modification in the duodenum. The SG procedure can cause remodification of the RNA epitranscriptome in duodenal tissue. The upregulation of this gene may play a significant role in the pathophysiology of the duodenal tissue after SG. Additionally, surgery can lead to pathological changes that may result in gastrointestinal tract diseases.

The limitations of this study include our focus on a relatively short post-operative period (six months), which prevents us from exploring the long-term effects of these epigenetic changes and their relationship to gastrointestinal diseases, including cancer. In addition, we were unable to perform comprehensive analyses to understand how these changes affect specific pathways, such as PI3K/Akt and mTOR signalling.

In conclusion, our findings in this study suggest the need for a comprehensive investigation of the RNA epitranscriptomic changes that occur in duodenal tissue after sleeve gastrectomy and to explore their broader pathophysiological implications. Firstly, further studies should be conducted to elucidate how m6A transcripts and other epitranscriptomic components change in gastrointestinal tissues after surgical intervention. In addition, mechanistic studies should focus on understanding the molecular and cellular changes induced by METTL14 gene upregulation in duodenal tissue. Long-term patient follow-up studies should be planned to assess the clinical significance of duodenal changes after sleeve gastrectomy. These studies could provide important insights into whether post-operative duodenal changes are associated with the development of digestive diseases and may help to identify potential treatment strategies. Finally, comparative studies should be carried out to assess whether the findings of this study are associated with different surgical procedures or metabolic conditions. This may contribute to a broader understanding of how similar changes may manifest in different contexts. All of these recommendations may provide an important foundation for understanding the fundamental mechanisms and clinical implications of epitranscriptomic changes in the gastrointestinal system.

Author Contributions: Concept/Design : BA, HS, SA; Data acquisition: CHT, BA; Data analysis and interpretation: CHT, BA; Drafting manuscript: CHT, BA; Critical revision of manuscript: : HS, CHT; Final approval and accountability: BA, HS, MEA, SA, DS, CHT; Technical or material support: BA, DS, HS, MEA; Supervision: BA; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was received from the Mersin University Clinical Research Ethics Committee (Approval number: 2022/213).
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

REFERENCES

1. Zhai Z, Li G, Tao Y, Wang Z, Han J. Sleeve gastrectomy plus uncut jejunojejunal bypass for the treatment of obesity and type 2 diabetes. Chin Med J. 2022;135:2240-1.
2. Gagner M. Effect of sleeve gastrectomy on type 2 diabetes as an alternative to Roux-en-Y gastric bypass: a better long-term strategy. Surg Obes Relat Dis. 2015;11:1280-1.
3. Andrea GI, Ana BC. Obesity-related epigenetic changes after bariatric surgery. Front Endocrinol. 2019;10:232.
4. González MM, Gracia MMN, Lourdes GS, Eduardo GF, Francisco T, Sonsoles M. Decreased blood pressure is related to changes in NF-kB promoter methylation levels after bariatric surgery. Surg Obes Relat Dis. 2018;14:1327-34.
5. Assem S, Abdelbaki TN, Mohy-E Dine SH, Ketat AF, Abdelmonsif DA. Serpine-1 gene methylation and protein as molecular predictors of laparoscopic sleeve gastrectomy outcome. Obes Surg. 2020;30:2620–30.
6. Beisani M, Pappa S, Moreno P, Martínez E, Tarasco J, Granada ML et al. Laparoscopic sleeve gastrectomy induces molecular changes in peripheral white blood cell. Clin Nutr. 2020;39:592-98.
7. He L, Li J, Wang X, Ying Y, Xie H, Yan H et al. The dual role of N6-methyladenosine modification of RNAs is involved in human cancers. J Cell Mol Med. 2018;22:4630–9.
8. Batista PJ. The RNA modification N (6)-methyladenosine and Its implications in human disease. Genomics Proteomics Bioinform. 2017;15:154–63.
9. Xie B, Deng Z, Pan Y, Fu C, Fan S, Tao Y. Post-transcriptional regulation DPC4 gene by miR-190 in colorectal cancer cells. J Cancer Res Ther. 2018;14:838–43.
10. Li J, Meng S, Xu M, Wang S, He L, Xu X et al. Downregulation of N (6)-methyladenosine binding YTHDF2 protein mediated by miR-493-3p suppresses prostate cancer by elevating N (6)-methyladenosine levels. Oncotarget. 2018;9:3752–64.
11. Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ et al. Mammalian WTAP is a regulatory subunit of the RNA N6- methyladenosine methyltransferase. Cell Res. 2014;24:177-89.
12. Xu L, Pan J, Pan H. Construction and validation of an m6A RNA methylation regulators based prognostic signature for esophageal cancer. Cancer Manag Res. 2020;12:5385–94.
13. Xia TL, Yan SM, Yuan L, Zeng MS. Upregulation of METTL3 expression predicts poor prognosis in patients with esophageal squamous cell carcinoma. Cancer Manag Res. 2020;12:5729–37.
14. Sang L, Sun L, Wang A, Zhang H, Yuan Y. The N6-Methyladenosine features of mRNA and aberrant expression of m6A modified genes in gastric cancer and their potential impact on the risk and prognosis. Front Genet. 2020;11:561-66.
15. Shen X, Zhao K, Xu L, Cheng G, Zhu J, Gan L et al. YTHDF2 Inhibits gastric cancer cell growth by regulating FOXC2 signaling pathway. Front Genet. 2021;11:592042.
16. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ et al. YTHDF3 facilitates translation and decay of N (6)-methyladenosine-modified RNA. Cell Res. 2017;27:315–28.
17. Zhao Q, Zhao Y, Hu W, Zhang Y, Wu X, Lu J et al. m6A RNA modification modulates PI3K/Akt/mTOR signal pathway gastrointestinal cancer. Theranostics. 2020;10:9528-43.
18. Cohen J. Statistical power analysis for the behavioral sciences. New York, NY: Routledge Academic, 1988.
19. Taylor MA, Szczerbinski L, Citko A, Niemira M, Gorska M, Hady HR et al. Sex-specific glucose homeostasis and anthropometric responses to sleeve gastrectomy in obese patients. Nutrients. 2019;11:2408.
20. Dilimulati D, Cai M, Lin Z, Zhang Y, Du L, Zhou D et al. Correlation between sex hormones and non alcoholic fatty liver disease before and after laparoscopic sleeve gastrectomy. Obesity Surgery. 2021;31:4901–10.
21. Gu L, Lin K, Du N, Minyao D, Lou D, Chen P. Differences in the effects of laparoscopic sleeve gastrectomy and laparoscopic Roux-en-Y gastric bypass on gut hormones: systematic and meta-analysis. Surg Obes Relat Dis. 2021;17:444-55.
22. Thomas RM, Jirapinyo P, Thompson CC. Effect of sleeve gastrectomy on ghrelin, GLP-1, PYY, and GIP gut hormones. Ann Surg. 2020;272:72-80.
23. Mackenzie H, Markar SR, Askari A, Faiz O, Hull M, Purkayastha S. et al. Obesity surgery and risk of cancer. Br J Surg. 2018;105:1650–1657.
24. Aravani A, Downing A, Thomas JD, Lagergren J, Morris EJA, Hull MA. Obesity surgery and risk of colorectal and other obesity-related cancers: An English population-based cohort study. Cancer Epidemiol. 2018;53:99–104.
25. Benvenga R, Roussel J, Cohen R, Bouchoucha M, Bendacha Y, Catheline JM. Long-term endoscopic follow-up after sleeve gastrectomy. J Visc Surg. 2022;159:39-42.
26. Shi B, Liu W, Yang K, Jiang G, Wang H. The role, mechanism, and application of rna methyltransferase mettl14 in gastrointestinal cancer. Mol Cancer. 2022;21:163.
27. Hu J, Lin H, Wang W, Su Q, Cao B. Mettl14-mediated rna methylation in digestive system tumors. Int J Mol Med. 2023;52:86.
28. Chen X, Xu M, Xu X, Zeng K, Liu X, Sun L et al. METTL14 suppresses CRC progression via regulating N6-methyladenosine-dependent primary miR-375 processing. Mol Ther. 2020;28:599–612.

29. Chen X, Xu M, Xu X, Zeng K, Liu X, Pan B, et al. METTL14-mediated N6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer. Mol Cancer. 2020;19:106.
30. Wang S, Gan M, Chen C, Zhang Y, Kong J, Zhang H et al. Methyl CpG binding protein 2 promotes colorectal cancer metastasis by regulating N(6)-methyladenosine methylation through methyltransferase-like 14. Cancer Sci. 2021;112:3243–54.
31. Liu X, Xiao M, Zhang L, Li L, Zhu G, Shen E et al. The m6A methyltransferase METTL14 inhibits the proliferation, migration, and invasion of gastric cancer by regulating the PI3K/AKT/mTOR signaling pathway. J Clin Lab Anal. 2021;35:e23655.
32. Zhang C, Zhang M, Ge S, Huang W, Lin X, Gao J et al. Reduced m6A modification predicts malignant phenotypes and augmented Wnt/PI3K-Akt signaling in gastric cancer. Cancer Med. 2019;8:4766–81.
33. Wang H, Wei W, Zhang ZY, Liu Y, Shi B, Zhong W et al. TCF4 and HuR mediated-METTL14 suppresses dissemination of colorectal cancer via N6-methyladenosine-dependent silencing of ARRDC4. Cell Death Dis. 2021;13:3.
34. Fan HN, Chen ZY, Chen XY, Chen M, Yi YC, Zhu JS et al. METTL14-mediated m(6)A modification of circORC5 suppresses gastric cancer progression by regulating miR-30c-2-3p/AKT1S1 axis. Mol Cancer. 2022;21:51.
35. Sorci M, Ianniello Z, Cruciani S, Larivera S, Ginistrelli LC, Capuano E et al. METTL3 regulates WTAP protein homeostasis. Cell Death Dis. 2018;9:796.