Original research

Effects of laparoscopic sleeve gastrectomy on serum lncRNA levels in obese patients

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Abstract

Obesity is a disease associated with excessive fat accumulation in the body, which body mass index (BMI) is ≥30 kg/m. Bariatric surgery technique is one of the most common treatment options for obesity with the advantage of faster weight loss in a short time. lncRNAs play a role in adipogenesis and metabolic diseases, including obesity, type 2 diabetes, cardiovascular disease (CVD), osteoarthritis, and hypertension, so they are significant targets for therapeutic options. In this study, we aimed to determine lncRNAs and specific parameters that show different expressions in the plasma of patients with obesity. We included fifteen patients with BMI ≥30 kg/m2 before and <30 kg/m2 after laparoscopic sleeve gastrectomy (LSG) in the study. Total RNAs, including lncRNAs and other non-coding RNAs, were isolated from plasma samples of patients, and eight lncRNAs (H19, Neat1, HOTAIR, ANRIL, MALAT1, ATB, SNGH5, UCA1) were quantified by real-time PCR. Gene Ontology, KEGG, and relation of obesity analysis were utilized. Unpaired Student's t-test Pearson correlation analysis was used for statistical analysis. We observed a statistically significant increase in the expression levels of all lncRNAs in the patients with the post-operative BMI change. We have added a new dimension to the biomarker studies related to obesity and the clinical follow-up of patients undergoing LSG surgery. Further studies are required for enlighting the molecular mechanisms.

Keywords: LSG surgery, lncRNA, Obesity, BMI

1. Introduction

In consequence of the requirement of modern lifestyle habits, obesity incidence has increased worldwide in recent years. World Health Organization identified that obesity is a disease associated with excessive fat accumulation in the body, linked to substantial risks for metabolic disorders and the pandemic process [1]. In diagnosis, body mass index is used worldwide [2], and a patient with body mass index (BMI) ≥30 kg/m is accepted as an obese person [1]. However, the current treatment option in the long term is ineffective depending on metabolic reactions robust homeostatic mechanisms that facilitate further weight gain [3]. Furthermore, BMI is an advisable parameter for diagnosing obesity, while having some critical limitations in diagnosing obesity at the individual level [4]. The BMI affects individual factors, including age, sex, and age-related factors, and it does not reflect body fat distribution [5]. Hence, there is a need for effective and specific biomarkers in early

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diagnosis. Subcutaneous and visceral adipose tissues are metabolically more active. They secrete cytokines and hormones that exert metabolic disturbances such as insulin resistance and chronic low-grade inflammation at a higher rate. Therefore, obesity is a disease processed by complex pathways [6].

One of the most common treatment options for obesity is bariatric surgery techniques, particularly laparoscopic procedures, as an effective treatment. Laparoscopic sleeve gastrectomy (LSG) is one of the most common bariatric operations that effectively treat obesity and severe obesity-associated diseases. LSG is a surgery method performed by removing approximately 80% of the lateral aspect of the stomach vertically. Studies reported that LSG has many advantages involving faster weight loss in a short time no foreign material as a gastric band. However, LSG can cause complicated biological problems due to the change of many biochemical parameters in the body and rapid weight alteration [3].

Non-coding gene is a significant part of the genome that serve primarily as regulatory functions. Long non-coding RNAs (lncRNAs) are usually longer than 200 nucleotides that modulate gene expression [7] and demonstrate organ- and lineage-specific lncRNAs in the human body [8]. Furthermore, studies revealed that lncRNAs play roles in adipogenesis and metabolic diseases, including obesity, type 2 diabetes, cardiovascular disease (CVD), osteoarthritis, and hypertension [9]. Recently, increased results indicated that lncRNAs have participated in crucial cellular mechanisms in the pathogenesis of human metabolic diseases. Specific regulations of metabolically sensitive lncRNAs in mice have been demonstrated [10]. On the other hand, the association between the expression pattern of MEG3, H19, and obesity parameters, essential genes in adipose tissues from obese women have been reported [5].

Therefore, lncRNAs are significant targets for therapeutic options. However, no studies reveal the relationship between circulating lncRNAs and obesity parameters after alteration BMI index through LSG. Therefore, in our study, we established the effects and correlation of circulating lncRNAs associated with metabolic process on standard obesity parameters. Furthermore, we aimed to determine lncRNAs and specific parameters that show different expressions in the plasma of patients with obesity.

2. Materıals and Methods

2.1 Sample collection

The Ethics Committee approved all protocols used in this study of Pamukkale University Hospital with the number E-60116787-020-110884. Informed consent was taken from each patient applied for the study. Blood samples were collected between January 2020 and April 2021. We did not include individuals whose BMI values did not fall into the expected level in the study. Briefly, 15 patients with BMI ≥30 kg/m2 before LSG and < 30 kg/m2 after LSG were accepted for the study. The blood samples were collected in 5 ml K_2EDTA tubes three days before and six months after surgery. The plasma samples were prepared as follows: Blood samples were centrifuged at 1500 rpm for 10 minutes at +4°C. Serum was divided into 15 ml Falcon tubes and centrifuged at 1500 rpm for 15 min at +4°C. The collected plasma samples were stored at -80°C.

2.2 Molecular analysis

Total RNAs, including lncRNAs and other non-coding RNAs, were isolated by TRIzol LS Reagent (Invitrogen, USA) and RNeasy Mini Kit (Qiagen, Germany) to manufacturers' instructions. Spectrophotometric measurements were performed at 260, 280, and 230 nm using the NanoDrop instrument (Thermo, USA). 260/280 and 260/230 ratios were evaluated for purity and quality and observed as 1.8-2.1 and 1.8, respectively.

The cDNA synthesis reaction was performed using miScript II RT Kit (Qiagen, Germany). PCR reactions were set in the Sensequest qPCR instrument (SensoQuest GmbH, Germany) using the following protocol: 37°C for 60 minutes and 95°C for 5 minutes. cDNA samples were stored at -20°C for the next stages of the experiments.

Real-time quantitative PCR (qRT-PCR), reactions were prepared using 2x Magic Sybr mix (Procomcure Biotech, Austria) for the quantitative analysis of lncRNAs, including H19, Neat1, HOTAIR, ANRIL, MALAT1, ATB, SNGH5, UCA1 [11], and performed using CFX96 Biorad instrument. U6 small nuclear RNA was the internal control. The reaction was performed in 3 replicates for each sample. The fold change in the expression of the lncRNAs was calculated using the formula $2^{-\Delta\Delta Ct}$.

2.3 Investigate the Relationships between obesity and lncRNAs

Collected data were analyzed using the Graphpad Prism 8.0.1 program and the Excel-based PCR Array Data Analysis Software provided by the Qiagen Company. We utilized the GO analysis, the pathway analyses, and Disease-lncRNA association analysis according to Gene Set Enrichment Analysis (GSEA) (https://www.gsea-msigdb.org/gsea/index.jsp) and DAVID database (https://www.gsea-msigdb.org/gsea/index.jsp). In addition, the KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http://www.genome.ad.jp/kegg) with Fisher's exact test was also used to identify pathways with p <0.05. Finally, the evaluated enrichment score of the paths is determined as previously described [12].

2.4 Statistical analysis

The graphs, calculations, and statistical analyses were performed using GraphPad Prism software version 8.0.1 (GraphPad Software, San Diego, CA, USA). Unpaired Student's t-test was used for comparisons differentially expressions of lncRNAs in the obese patients after LSG operation. A receiver operating characteristic (ROC) curve was employed for each lncRNA. Their respective 95% confidence intervals (CI) were calculated to evaluate the sensitivity and specificity for obese patients. Pearson Correlation Coefficient analysis was utilized to determine the relationship between expression levels of lncRNAs and BMI index alteration. Statistical results with *p <0.05, **p <0.01, ***p <0.001, or ****p <0.0001, were considered to be statistically significant.

3. Results

Fifteen patients (n=12 women, n=3 men) who had undergone LSG were included in our study. The mean age of the patients was 34.13±13.23. The mean preoperative weight of the operated patients was 121.66±21.82, while the mean post-operative weight was 85.06±19.36 (p <0.0001). In addition, the mean BMI changes before and after the operation were 49.03±20.34 19.36±9.1, respectively. Besides, statistically significant differences were observed in the glucose, TSH, T3, CRP, and triglyceride levels after the surgical process (Table 1).

H19, Neat1, HOTAIR, ANRIL, MALAT1, ATB, SNGH5, and UCA1 lncRNA expression levels were investigated by qRT-PCR analysis from serum samples taken from patients two times as before and six months after the operation. qRT-PCR analysis results revealed a statistically significant increase in all lncRNA expressions in the samples six months after the operation (Figure 1). ATB and HOTAIR expression were regarded as the lncRNAs with the most statistically significant increase among all lncRNAs with increased expression. The data we obtained from the gene ontology analysis showed that lncRNAs play a role in obesity and related mechanisms (Supplementary Figure 1). When T3, glucose, TSH, CRP, and triglyceride levels were analyzed before and after the operation, it was observed that there was a blind decrease after the surgical operation. AUC values were above 0.8 for all analysis groups (Supplementary Figure 2).

When the lncRNA expression changes observed in the operated patients were analyzed according to the BMI changes, it was observed that there was an increase in the expression levels of all lncRNAs. Furthermore, these changes were statistically significant (Figure 2).

4. Discussion

LncRNAs are generally identified as transcripts longer than 200 nucleotides compared to types of short RNA such as miRNAs, siRNAs, and piRNAs [9]. They display different expression patterns according to tissues and conditions in the human body. Moreover, their variable expression responds to numerous stimuli and timely expression at specific developmental requirements [10]. LncRNAs are being widely studied to play a role in adipogenesis and metabolic diseases and obesity and obesity-associated as type 2 diabetes, and CVD [13]. Furthermore, lncRNAs have been reported to affect the chronic positive energy balance, including overeating and low energy expenditure [1, 13], but new studies are required in this area. Therefore, in the present study, we investigated the relationship between the potential alteration of circulating lncRNA and the physical and biochemical parameters of obesity after LSG. Lately, there have been many experiments and research related to the role of lncRNAs in various diseases and mechanisms. In contrast, the action of the vast majority of lncRNAs remains unclear [10].

With the advancements of high-throughput techniques, transcriptome data of lncRNAs possesses an enormous amount of information related to

Table 1. The clinical characteristic feature of patient group

Variable	Group Total n = 15		Significant level
Age (years)	34.13±13.23		
Gender	12, female	3, male	
Height (cm)	161.93±15.61		
Surgery condition	**Before**	**After**	**P value**
Weight (kg)	121.66±21.82	85.06±19.36	P <0.0001
BMI	49.03±20.34	19.36±9.1	P <0.0001
Glucose (mmol/L)	122.06±82.05	81.2±8.38	P <0.0001
TSH (mU/L)	3.46±2.65	2.09±0.91	P <0.0001
T3 (nmol/L)	3.41±0.51	2.55±0.39	P <0.0001
CRP	0.9±0.65	0.65±0.84	P <0.0001
Triglyceride (mmol/L)	167.92±105.84	96.84±24.34	P <0.0001

*The P values refer to comparisons between "Before surgery" and "After surgery" groups in the top part of the table.
**Data are represented as mean ± SD.
***BMI; Body mass index, TSH; Thyroid stimulating hormone, T3; Triiodothyronine.

patients with metabolic diseases such as obesity [7]. The results obtained from studies consist of a combination of RNA-seq, and computational methods that are currently working to identify novel candidate lncRNAs as potential biomarkers and possible therapeutic targets [14]. Therefore, Sun et al. demonstrated that the expression pattern of three lncRNAs is different in the blood components and adipose tissue of obese patients against the control group, including non-obese persons [14]. However, there is a deficiency in the correlation between expression alteration of lncRNAs and obesity-related parameters.

On the other hand, Yao et al. described lncRNAs as potential biomarkers in metabolic syndrome [6]. In the same direction as previous studies, in our research, we determined a correlation between alteration in the expression pattern of especial lncRNAs and metabolic parameters of obesity in the patients the transition from obese to non-obese according to the BMI index. Moreover, our results indicated that lncRNA H19, Neat1, HOTAIR, MALAT1, ATB, UCA1, and SNGH5 correlate with alteration of BMI index. Thus factors

can influence them in energy metabolism and obesity-associated physical activity; there is a need for elucidating to functional roles of these lncRNAs in both *in vitro* and *in vivo* studies. Current studies showed the role of lncRNAs in obesity-associated biological mechanisms, including adipogenesis (lncRNA H19, Neat1) [15], lipid metabolism (Neat1) [16], white adipogenesis (HOTAIR), and adipose regulation (MALAT1) [17]. Furthermore, in the present study, KEGG pathway enrichment results and Pearson correlation analysis indicated that lncRNA ANRIL, ATB, UCA1, and SNGH5 and these lncRNAs mentioned in the literature have the potential to be biomarkers in the follow-up of obese patients after LSG. To study the disease relation and biological functions of these lncRNAs, we utilized the GO analysis, the pathway analyses, and disease-lncRNA association analysis by working GSEA and DAVID database. These analysis results demonstrated that these lncRNAs play an essential role in the obesity and disease linked to obesity, including diabetes mellitus, insulin resistance, and hypertension. Furthermore, we showed connections between lncRNAs including H19,
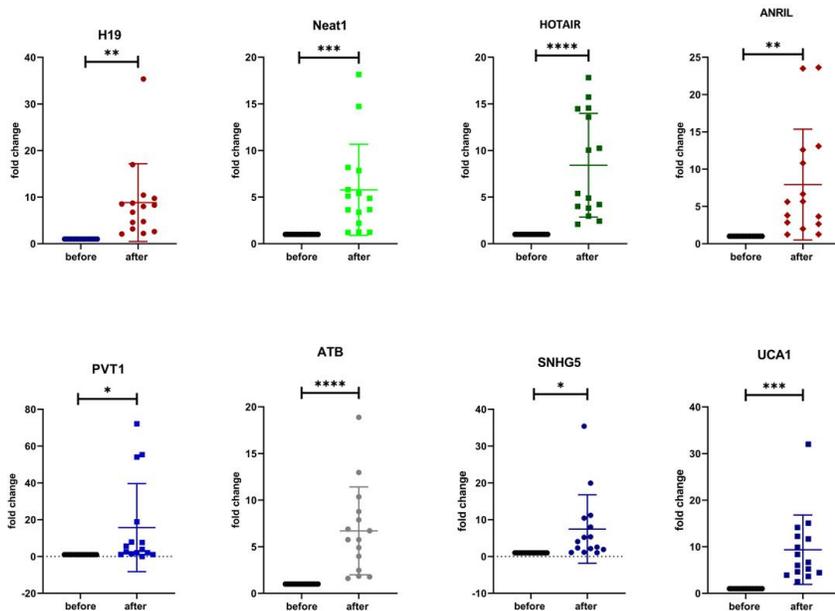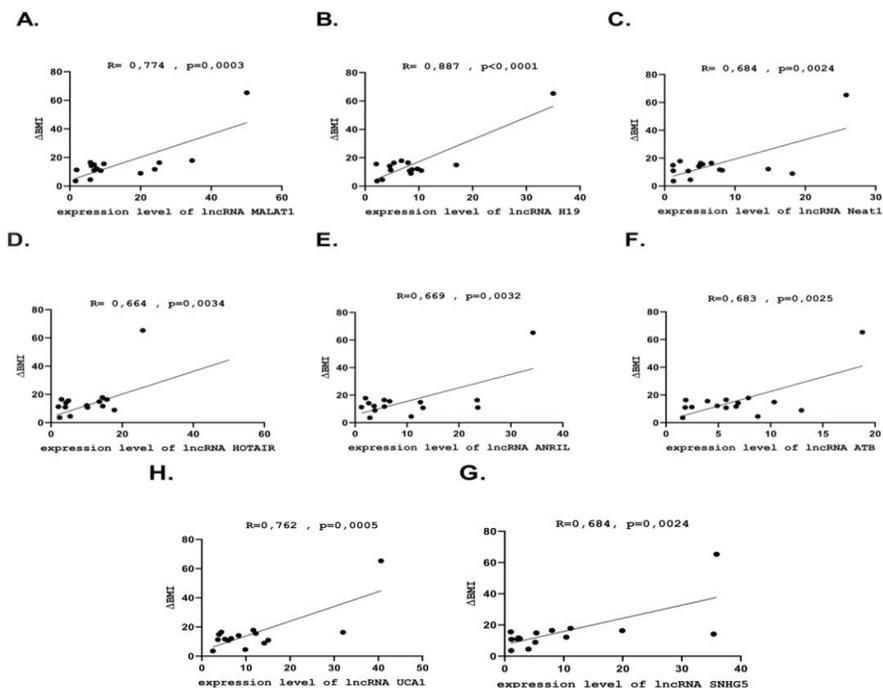
Figure 1. Expression level of circulating H19, Neat1, HOTAIR, ANRIL, MALAT1, ATB, SNGH5, UCA1 in before and after surgery. Expression of lncRNAs were detected by qPCR and normalized by U6 expression. (*p <0.05, **p <0.01, ***p <0.001, or ****p <0.0001).

Figure 4. Correlation of MALAT1 (A), H19 (B), Neat1 (C), HOTAIR (D), ANRIL (E), ATB (F), UCA1 (G) and SNGH5 (H) expressions with ΔBMI (ΔBMI:preBMI-postBMI).

Neat1, HOTAIR, MALAT1, ATB, UCA1, SNGH5, and genes in the obesity-associated pathways. Consistent with our findings, a study on the obese women group reported that lncRNA MEG3 and H19 were associated with a metabolic profile in the adipose tissue of obese women.

Obesity is an epidemic factor for several chronic diseases involving type 2 diabetes, coronary heart disease, and different types of cancer [4]. Epidemiological studies demonstrated that classical anthropometric measures including height, weight, head circumference, and BMI are used to identify obesity. However, the underlying biological mechanisms are less understood as obesity is a complex disease including biochemical and genetic factors. The current knowledge of the significant roles

of obesity-related biomarkers in disease development is inadequate [18, 19]. An increasing number of epidemiological studies have investigated the relationship between biomarkers associated with obesity and disease risk. Therefore, there is a needing to discover consistent and reliable biomarkers associated with obesity and changing biochemical parameters [19-21]. Even though many investigations have been continued in metabolomics, epigenetic, transcriptomic, and proteomic studies are also required.

Together with the present study, we have added a new dimension to the biomarkers related to obesity and the clinical follow-up of patients undergoing LSG surgery. However, we have realized that our research has several limitations, including a larger sample cohort that further verifies in the broader working group elucidation of molecular mechanisms that relate potential lncRNAs and associated pathways. Together with our study, we present new research topics in the literature to illuminate these blind spots.

Supplementary files
Supplementary file 1.

Authors' contributions
Study Conception: OT, ACD, OB; Study Design: OT, KI, BC, PET; Supervision: OT, OB, ACD; Materials: OB; Data Collection and/or Processing: OT, KI, BC, PET; Statistical Analysis and/or Data Interpretation: OT, KI; Literature Review: OT, KI, BC; Manuscript Preparation: OT, KI, PET, and Critical Review: OT. All authors read and approved the final version of manuscript.

Conflict of interests
The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Ethical declarations
The Ethics Committee approved all protocols used in this study of Pamukkale University Hospital with the number E-60116787-020-110884. Informed consent was taken from each patient applied for the study.

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