

ORIGINAL ARTICLE

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The function of obesity related miR223-3p as a potential biomarker for weight loss prediction following bariatric surgery

¹Samet Yigman¹, ²Beyza Goncu², ³Suleyman Bozkurt³, ⁴Yunus Yapalak³, ⁵Ahmet Necati Sanli⁴,
⁶Kubilay Oztutuncu⁵, ⁷Halil Coskun³, ⁸Erkan Yardimci³

¹Koc University Hospital, Department of General Surgery, Istanbul, Türkiye

²Bezmialem Vakif University, Experimental Research Center, Istanbul, Türkiye

³Bezmialem Vakif University, Faculty of Medicine, Department of General Surgery, Istanbul, Türkiye

⁴Abdulkadir Yuksel State Hospital, Department of General Surgery, Gaziantep, Türkiye

⁵Eyupsultan State Hospital, Department of General Surgery, Istanbul, Türkiye

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Abstract

The objective was to determine how miRNA expression levels related to obesity and weight loss interacted, to forecast how much weight patients could lose after surgery, and to maximize the advantages of a scheduled procedure. 17 patients who planned laparoscopic sleeve gastrectomy were included. Demographic variables, comorbid conditions, and postoperative excess weight loss rates (EWL%) were evaluated. Both intraoperative adipose tissue samples and blood samples were taken. The gene expression levels of the miRNAs (miR27b-3p, miR122-5p, and miR223-3p) linked to diabetes and obesity were investigated. Mean body mass index was 49.17.6 kg/m². Six individuals had diabetes mellitus, and their mean blood sugar and HbA1c levels were 133±59.4 mg/dl and 6.4±1.3%, respectively. All preoperative groups had considerably higher levels of miR223-3p expression, it was discovered. Blood samples taken before and after surgery showed considerably higher levels of miR223-3p gene expression than those of miR27b-3p and miR122-5p. Additionally, it was discovered that in individuals with diabetes mellitus, miR122-5p gene expression in fatty tissue was lower than that of miR223-3p and miR27b-3p. MiR223-3p expression has been linked to morbidly obese patients, particularly those with EWLs of 50% and above. After bariatric surgery, the miR223-3p gene may be utilized as a potential biomarker to predict a patient's capacity to lose weight. It is possible to identify patients who will not benefit from surgery and decide to perform a different kind of operation on them. However, additional research involving more patients, additional miRNAs, and various bariatric surgery techniques is required.

Keywords: Obesity, miR223-3p, biomarker, bariatric surgery

Introduction

Obesity is an important health problem, especially with the recent frequency increasing all over the world [1]. The increase in obesity prevalence shows the importance of accurate definition and appropriate treatment of obesity. The body mass index (BMI) is the main diagnostic criteria to determine the appropriate treatment option. Treatment options such as diet, exercise, lifestyle modification, pharmacological treatments,

endoscopic procedures and bariatric surgery are available in the treatment of obesity, with bariatric surgery accepted as the most effective treatment method [2]. However, patients undergoing surgical treatment may differ in terms of weight loss rates. The elevated levels could be linked to a variety of clinical problems such as age, diet compliance, distribution of pre-operative body composition, exercise, and the type of surgery completed, but a specific cause has not been determined yet [3-5].

CITATION

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Corresponding Author: Ahmet Necati Sanli, Abdulkadir Yuksel State Hospital, Department of General Surgery, Gaziantep, Türkiye
Email: ahmetnecatisanli@gmail.com

Current strategies revealed the importance of how the genetic composition of individuals such as miRNA levels have been shown to play a role in this regard. By binding to diverse regions of mRNAs, miRNAs suppress the expression of numerous targets and play a significant role in the regulation of many biological processes. Recent research has demonstrated the critical functions that miRNAs play in the development, differentiation, death, immunology, and angiogenesis of target cells that contain target genes [6]. Changes in specific miRNA expression levels have been reported in several diseases such as diabetes mellitus (DM), hyperlipidemia, malignant diseases, and obesity. Several diseases have been shown to be associated with certain specific miRNAs [5,7,8]. Many studies have shown that many miRNAs such as miR27, miR223, miR224, miR935, miR4772, miR126, miR122, and miR143 are associated with metabolic diseases such as obesity and Type II DM [9,10].

Type of surgery plays a major role in the factors affecting weight loss rate, particularly within the first six-months post-operatively. Considerable attempts have been made to improve the relationship between several miRNAs and with obesity or weight loss for the same time interval. Therefore, most of the studies that use how genetic factors are affecting and have shown differential miRNA expression levels may correlate with obesity and some metabolic diseases [11,13].

This study aims to determine the effect of particular miRNAs including miR27b-3p, miR122-5p, and miR223-3p which were evaluated pre- and post-operatively from blood samples and compared with adipose tissue expression profiles in patients undergoing bariatric surgery.

Material and Methods

This prospective study was approved by the local Ethical Board of Bezmialem University (approval number: 71306642-050.01.04-18464). Between May 2017 and July 2018, 17 patients who were planned for laparoscopic sleeve gastrectomy (LSG) were included in the study, and the patient's data were analyzed prospectively.

The study comprised patients who had LSG and had a BMI above 40 kg/m² or a BMI over 35 kg/m² coupled with at least one obesity-related condition. Patients with prior stomach surgery (gastric plication, adjustable gastric banding, hiatal hernia repair, and gastric fundoplication) were excluded, as were those with osteoarticular disease that would limit exercise, cancer, and ages between 18 and 65.

Demographic characteristics of the patients (age, gender, BMI (kg/m²), body fat composition), and comorbid diseases were collected. One gram samples of adipose tissue were collected intraoperatively, as well as preoperative and postoperative six-month venous blood samples. The adipose tissue samples were placed in the "RNA later solution" and the blood samples were placed in the DNA/RNA Shield Blood Collection Tube. All samples taken were enumerated and classified and stored in the

freezer at -80°C following the cold chain rule and these samples were stored at -80°C until use. The samples were tested for the expression of the miR27b-3p, miR122-5p, and miR223-3p genes linked to obesity. The obesity-related miR223-3p, miR27b-3p, and miR122-5p were determined and relative expressions were calculated.

All patients' body fat compositions were measured and BMI and EWL% were calculated at the postoperative six-month check-up. Based on the patients' rates of weight loss as measured by EWL% values, the patients were split into three groups: EWL% value as 0-50 % (Group-1), 50-70% (Group-2), and 70-90% (Group-3). It was investigated that the differences in miRNA expression levels between the groups. All operations were performed laparoscopically by two bariatric surgeons and there was no intra-operative or post-operative complication.

Total RNA isolation and cDNA synthesis

Following the manufacturer's instructions, a 500 µL blood sample was utilized for total RNA isolation using the commercial kit, and the collection tubes were incubated on ice for a thorough thawing procedure (Total RNA Purification Kit, Norgen, Ontario, Canada). The concentration and quality of isolated total RNA samples were determined by using Multiskan GO (Thermo Fisher Scientific, MA, USA). The ratio of 260nm and 280nm between 1.8 and 2.1 were assumed acceptable. Then, the RNA concentration of each sample was equalized to 500 ng/µL. After that, cDNA synthesis was performed. In a summary, the miScript II RT Kit (Qiagen, Hilden, Germany) was used to perform reverse transcription of 500 ng total RNA on a total of 51 samples (17 blood samples from pre- and post-operative and 17 adipose tissue samples). For later analysis, the acquired cDNAs were kept in a freezer at -20°C.

Stem-loop RT-PCR: Using the miScript SYBR Green PCR Kit and miScript Primer Assay (both from Qiagen, Hilden, Germany), real-time PCR for miRNAs was carried out on a CFX-connect Real-Time PCR Instrument (Biorad, CA, USA) in accordance with the manufacturer's instructions. RNU6-2 (RNA, U6 small nuclear 6, pseudogene, Entrez Gene ID: 26826, Transcript (s): NR002752) was employed as an endogenous reference control. The miRNAs were identified as hsa-miR-27b-3p, hsa-miR-122-5p, and hsa-miR-223-5p by miRBase (MIMAT0000419) and MIMAT0000421, respectively (miRBase Accession: MIMAT000028). Using the 2-ΔΔ CT approach, relative miRNA expression levels were determined [14].

Statistical Analysis

Before collecting samples, the power analysis was performed and when we accepted the baseline correlation on 0.85, the sample size for 95% confidence level and 80% power at α=0.05 significance level is calculated as n=6 per variable. miRNA relative expressions were calculated according to the delta-delta Ct method and statistical analysis was performed by GraphPad interface (Prism 8.4, GraphPad Software, Inc., CA, USA). Data were compared by the Mann – Whitney U test and, Sidak's

multiple comparisons test. A p-value <0.05 was considered to be statistically significant.

Results

Four of the patients were male (23.5%), 13 were female (76.4%), the average age of 41±11.6 years, and the average BMI was 49.1±7.6 kg/m2. Six patients (35.2%) had diabetes mellitus, and their mean blood glucose and HbA1c% values were 133.59.4 mg/dl and 6.4 1.3%, respectively. The average EWL% rate at the six-month postoperative follow-up was 62.717%. The demographic variables of the patients are presented in Table 1.

Table 1. Demographic variables of the patients			
Features	Group-1 n=4	Group-2 n=7	Group-3 n=6
Average levels			
Age	38.5±11.3	43.5±13.2	41.3±11.6
Sex (Male/Female)	2/2	2/5	0/6
BMI (kg/m2)	52.4±7.4	46±4.5	43.5±3.2
EWL %	%41.2±6.1	61.9±4.2	79.6±7.9
HbA1c %	%6.4±1.2	5.7±0.3	5.6±0.3
Diabetes Mellitus	3/4	2/7	1/6
Glucose (mg/dl)	132±54.8	109.7±41.8	92.6±21.3

In all pre-operative blood samples from each of the three groups, relative expression levels of miR223-3p were found to be greater (p = 0.0046 for Group-1; p 0.0001 for Group-2; and p = 0.0017 for Group-3, respectively). Additionally, miR223-3p relative gene expression level changes were discovered to be significantly higher than miR27b-3p and miR122-5p in pre- and post-operative blood samples from Groups 2 and 3 (p values for Group 2 are p = 0.0335 and p = 0.0328, respectively, and p values for Group 3 are p=0.0446 and p = 0.0441 pre- and post-operative, respectively) (Figure 1). The miR27b-3p was not found significant for pre- and post-operative blood samples (p=0.7001).

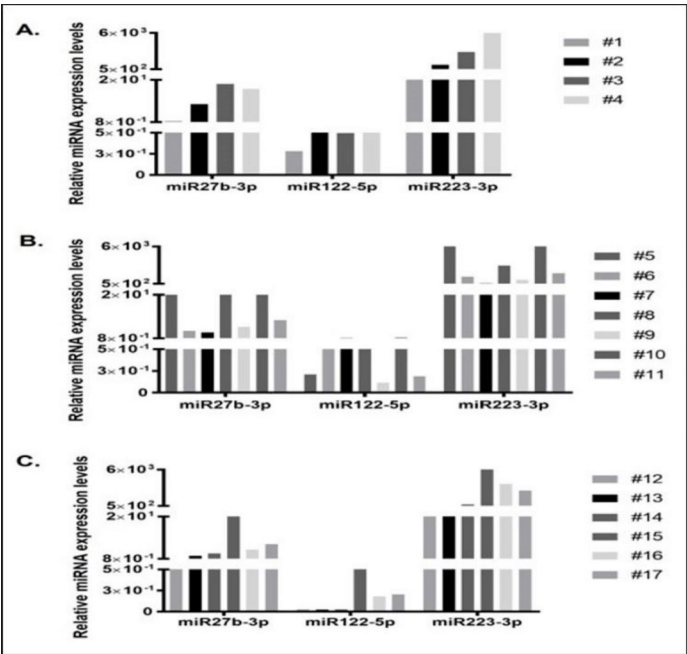


Figure 1. miRNA expression changes in pre-operative and post-operative blood samples of patients

The relative miRNA expressions were compared in both groups. In Group-1 and Group-3 preoperative adipose tissue miRNA expression was found significantly decreased for miR122-5p (p=0.0118 and p=0.0453 respectively). On the other hand, in Group-2 preoperative adipose tissue relative miRNA expression was not significant.

We further evaluated DM-negative and positive patients' adipose tissue miRNA expression levels. The miR27b-3p, miR122-5p and miR223-3p expression levels in preoperative tissue samples were not significantly different between DM-negative patients, however, the expression levels are miR223-3p>miR37b-3p>miR122-5p respectively. On the one hand, when compared with DM-positive adipose tissue samples, we only found that miR27b-3p was significantly higher than miR122-5p (p=0.0112). On the other hand, patients with diabetes mellitus had lower levels of miR122-5p relative miRNA expression in adipose tissue than those without the disease (p=0.0062) (Figure 2).

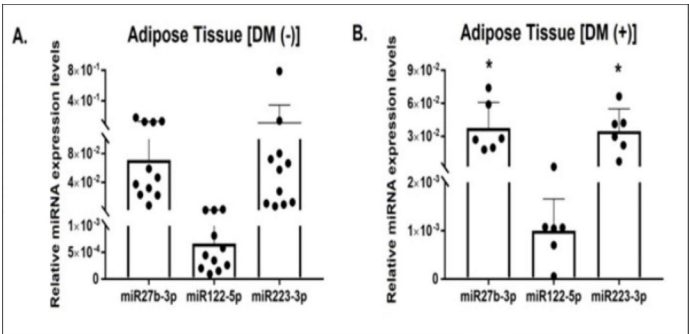


Figure 2. miRNA gene expression changes in adipose tissue of patients with/without type II diabetes mellitus (DM)

Discussion

The higher rate of obesity has become a global health issue that has been largely attributed to the increasing number of people diagnosed with it. Due to the increasing number of surgical procedures being performed on obese individuals, the treatment of this condition has become the most effective therapy [15]. The role of certain types of microRNAs in gene expression has also been recognized. These are known to play a role in the development of various diseases such as diabetes and cancer [16]. It was designed to investigate the association between this variation and obesity-specific miRNAs because the postoperative weight loss rates of individuals who underwent bariatric surgery vary amongst people. In order to maximize the benefits of the planned surgery and forecast the postoperative weight reduction of patients who are preparing to have bariatric surgery, a relationship between the two variables is therefore sought after.

In a multicenter study by Willeit et al., various miRNA expressions were examined in blood samples from more than 2000 people based on the normal population to identify the metabolic pathways associated with miR122-5p, and the miR122-5p gene expression levels that may occur in the general population with

future metabolic syndrome and Type II DM [17]. MiR122-5p is considered to be a biomarker for obesity and weight loss in this regard. The miR122-5p level detected in blood tissue samples was significantly greater in individuals who underwent bariatric surgery as a result of obesity, and there was a significant positive link between the miR122-5p level and BMI and lubrication, according to the research done by Ortega et al. After multivariate regression analysis, it was revealed that it was not an independent determinant of obesity except for brown adipose tissue activity for miR122-5p [18]. Therefore, the correlation between the miR122-5p level and obesity previously reported in some studies is thought to be largely due to brown adipose tissue activation rather than the degree of BMI. In our study, it was observed that there was no significant relationship between weight loss when relative expression results for miR122-5p were compared preoperatively and postoperatively.

In an in vivo study by Zhuang et al., it was shown that miR223-3p was significantly higher in adipose tissue in mice that were fed with a high-fat diet compared to mice fed with a normal diet [19]. In contrast, Zampetaki et al. showed lower miR223-3p expression levels were found in obese patients when compared with miR454; and miR223-3p levels were thought to be effective in predicting the disease in patients with obesity and Type II DM [20]. In another study by Wen D. et al., three months of lifestyle change increased serum miR223-3p expression in overweight and obese patients. Therefore, it was stated that miR223-3p expression could be used as a biomarker in the prediction of obesity [21].

In line with our findings, studies from the literature about miRNA expression have increased significantly, with the realization of the effects they have on many diseases such as diabetes mellitus, hyperlipidemia, malignant diseases, and obesity. In our study, compared to miR27b-3p and miR122-5p expressions, the postoperative EWL% rate was higher than 50% in morbidly obese patients with high expression of miR223-3p. In this situation, a potential biomarker for predicting weight loss following bariatric surgery is the miR223-3p gene. As a result, the surgical procedure will be more effective and the group of individuals who will benefit from it the most can be identified beforehand. The surgical strategy can be changed from restrictive surgical procedures to mixed (restrictive + malabsorptive) or malabsorptive surgical procedures at the same time as individuals that will not benefit from planned surgery can be identified. These results have been suggesting that different miRNAs can potentially be used in predicting progress toward metabolic diseases. These findings would improve the current therapeutic strategies and diagnostic effectiveness of obesity. For this, further studies are needed that include more patient groups, more miRNA types examined in different tissues, long-term follow-up, and different bariatric surgery procedures compared. This study has some limitations due to fewer patient numbers, examination of limited miRNA subgroups, and a relatively a short follow-up period.

Conclusion

In conclusion, it has been demonstrated that patients who are morbidly obese, especially those with an EWL of 50% or more, have high expression levels of miR223-3p. A potential biomarker for predicting weight loss following bariatric surgery is the miR223-3p gene. Additionally, groups that won't benefit from surgery can be found, and a choice can be made to change the surgery's type. However, more research is required with a larger sample size, more miRNA types, and a comparison of various bariatric surgery techniques.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

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Ethical approval

The study protocol was approved by Bezmialem University Ethics Committee (approval number: 71306642-050.01.04-18464).

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