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Expressions of Micro-RNAs 365 and 375 in Obese Individuals suffering from Type 2 Diabetes

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Abstract

Background: Obesity and type-2 diabetes (T2D) are two interconnected public health challenges that have reached epidemic proportions worldwide. Both conditions are characterized by deregulated metabolism. We have a poor knowledge of the fundamental mechanisms underlying these health issues. MicroRNAs (miR) have recently emerged as crucial contributors in the pathophysiology of several metabolic diseases. Among microRNAs, miR-365 and miR-375 have received attention for their possible roles in obesity and T2D. The purpose of this study was to investigate the expression patterns of miR 365 and 375 in obese adults with and without T2D using quantitative real time polymerase chain reaction (qRT-PCR), in order to shed light on their potential linkages to these disorders. **Results:** MiR-365 is up-regulated in obese with or without T2D. Conversely, miR-375 expression was significantly down-regulated in obese patients without T2D, implying a possible role in impaired insulin secretion and beta-cell dysfunction, whereas miR-375 expression was persistently reduced in obese patients with T2D, potentially contributing to beta-cell dysfunction and impaired insulin secretion. In terms of clinical parameters, miR-365 was associated with both cholesterol and low-density lipoprotein levels, but miR-375 was associated with only high-density lipoprotein levels. **Conclusion:** MiR-365 and miR-375 have unique expression patterns in obese people with and without T2D, indicating that they are involved in the molecular pathways driving these metabolic diseases.

Keywords: MiR-375; MiR-365; Obesity; Type 2 diabetes; biomarker.

1. INTRODUCTION

Type 2 diabetes (T2D) and obesity are common metabolic illnesses that are characterized by deregulated metabolism, insulin resistance, and persistent low-grade inflammation [1]. Obesity is a systemic sickness and a well-known contributing factor for T2D metabolic disorders, but our present understanding of the underlying fundamental mechanisms is limited [2, 3]. When macrophages and T cells, for example, infiltrate adipose tissues, they release pro-inflammatory mediators that lead to insulin resistance. Obesity caused persistent low grade inflammation and the onset of the "auto inflammatory disease" metabolic syndrome [4]. During disease progression, inflammatory activity causes glucotoxicity, lipotoxicity, oxidative damage, and endoplasmic reticulum stress [5]. Chronic inflammation is commonly related to the occurrence of insulin resistance, pancreatic islet B cell malfunction, and ultimately T2D due to higher systemic concentrations of pro-inflammatory cytokines and anti-inflammation downregulation [6]. MicroRNAs (miRNAs) are well-known non-protein coding RNAs that interfere with protein synthesis by either mRNA degradation or translation inhibition. This results in the regulation of cell differentiation, growth, death, and proliferation [7]. In summary, via base-pairing with their target mRNAs, this family of

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short RNAs acts as post-transcriptional regulators of gene expression. Each microRNA could target several mRNAs, and a single transcript might incorporate multiple miRNA binding sites [8]. MiRNAs are thought to impact more than 60% of protein-coding genes [9]. Circulating miRNAs are becoming widely recognised as potent biomarkers for human illnesses because they can signal the stimulation status of circulating cells or tissue damage in response to disorder conditions [10]. As a result, changed circulating miRNA levels have been observed at several stages of disease aetiology, such as obesity and type-2 diabetes [11]. Among these microrNAs, miR-375 and miR-365 have gotten a lot of interest because of their possible roles in obesity and T2D aetiology. Indeed, MiR-375, for example, is mainly expressed in pancreatic islet cells and regulates insulin production and pancreatic beta-cell activity [12]. MiR-375 expression has been found to be altered in the pancreatic islets and peripheral tissues of people with obesity or T2D. MiR-375 expression is often reduced in obese people, which may contribute to poor insulin secretion and beta-cell dysfunction [13]. MiR-375 has been found to target genes involved in insulin exocytosis, glucose metabolism, and beta-cell proliferation, highlighting its importance in maintaining pancreatic homeostasis [14]. In animal experiments, directly suppressing miR-375 in zebra fish produced substantial defects in pancreatic islet development. Mice with homozygous miR-375 deletion seemed to have hyperglycemia due to decreased insulin levels and total mass of B cells in the pancreas [15]. MiR-365, on the other hand, has been linked to numerous elements of metabolic control. Obesity has been linked to altered expression of miR-365 in adipose tissue and pancreatic islets [16]. MiR-365 expression is frequently up-regulated in obese people, and it has been linked to adipocyte differentiation, insulin resistance, and poor glucose metabolism. Over expression of miR-365 in adipose tissue causes insulin resistance and glucose intolerance, while inhibition improves insulin sensitivity [17]. The goal of this work was to look at the expression patterns of miR-365 and miR-375 in obese people with and without T2D, to be able to shed light on their potential molecular linkages to these disorders.

2. SUBJECTS AND METHODS

Study design and subjects

The present study enrolled 85 people with BMIs more than 30 kg/m2 (25 healthy donors, 30 obese without diabetes, and 30 obese with diabetes) who had bariatric surgery for obesity at the Surgery Unit. All participants provided written informed consent.

Data collection and exclusion criteria

We collected demographic information, clinical complaints, and laboratory results. Patients were ruled out if they had one or more of the following exclusion criteria: liver illness, renal disease, or a history of malignancy.

Specimens Collection

After a 12-hour fast, all patients had 5 mL of peripheral venous blood drawn. After allowing blood to coagulate at room temperature (25°C), it was centrifuged at 3000 xg for 10 minutes. Serum was split into two sections, one for biochemical analysis and one for RNA extraction using QIAZol, and kept at -80°C until miRNA expression levels were assessed.

Biochemical analysis

Serum total cholesterol (TC), triglyceride (TG), and HDL-c levels were calorimetrically tested using a Stanbio laboratory (USA) kits. Friedewald's an equation was used to compute LDL-c as TC-HDL-c-TG/5 [18].

MiRNAs expression assessing

Utilising the miRNeasy Mini isolation kit (QIAgen, Germany), total RNA was obtained as directed by the manufacturer. The concentration and purity of the RNA were assessed using the Thermo Fisher Scientific Inc. USA NanoDrop spectrophotometer®. A260/A280 ratio analysis demonstrated that all of the extracted RNA samples are of appropriate purity for qPCR assay (1.93-2.10). Following the manufacturer's instructions, particular miRNA reverse transcription (RT) primers and the microRNA Reverse Transcription Kit were used to reverse transcribed miR-375 and miR-365. For achieving a final volume of 20 μL, 2 μL of RT products were mixed with 10 μL of SYBR green PCR master mix, 1 μL miRNA assays, and nuclease-free water. The following conditions were applied to every reaction when it was conducted on a Rotor Gene Q real-time system: Ten minutes at 95 °C, followed by forty cycles of 15 seconds at 95 °C and 60 seconds at 60 °C. The relative expression of target miRNAs was normalized to MiR-U6. The equation $2^{-\Delta\Delta Ct}$ calculated the fold changes in the expression of potential miRNAs [19].

Statistical analysis

Employing SPSS version 20 (SPSS INC. Chicago, IL, USA), the data was analysed. Quantitative variables were described using their means, percentages, and standard errors. To compare groups, the analysis of variance ANOVA test was used. Correlations between miRNA expression levels and biochemical parameters were calculated using Spearman rank and Pearson correlation coefficients. The difference between groups was deemed statistically significant when $p < 0.05$ was used.

3. RESULTS

Demographic and clinical characteristics of the participants

The biochemical clinical characteristics of the study subjects are showed in Table 1 including control (n=25), obese without T2D (n=30), and obese with T2D (n=30) groups. We found that obese with T2D patients are assesed increased values of body mass index (BMI), triglycerides, fasting blood sugar (FBS), and insulin with statistically significant change compared to the control and obese without T2D groups (Table 2).

Likewise, obese without T2D patients showed statistically significant change compared to the control in BMI, Cholesterol, Triglycerides, FBS, Homo-IR and low density lipoprotein (LDL) cholesterol. Finally, the levels of urea and creatinine as well as levels of liver enzymes AST and ALT were similar among groups (Table 1) and have no statistical significant (Table 2).

Principal component and correlation analyses

A descriptive principal component analysis (PCA) was carried out to investigate the relationship between all the variables studied (Figure 1). The PCA showed the grouping pattern of the individuals by studied groups. Indeed, obese with or without T2D tended to cluster together. According to the factorial map for variables, the two microRNAs' expression levels tended to be uncorrelated with most of the biochemical parameters. Similar relationships were seen in both the PCA and the Pearson matrix correlation (Figure 1 & 2). There was a weak positive correlation between the miR-365 and miR-375. Both microRNAs were significant negatively correlated with the Homeostatic Model Assessment (HOMA) IR, cholesterol, and insulin (Figure 2). While, a positive association between insulin levels and the HOMA IR was seen.

Circulating miR-365 and miR-375 in serum

We assessed the expressions of the miR-365 and miR-375 in the sera of individuals with control, obese without T2D and with T2D patients. We found that miR-365's relative expression in obese without T2D and with T2D patients was up regulated in contrast with control group (Figure 3). Besides, the relative expression of miR-375 in obese patients either with or without T2D was persistent down regulated compared to the control group (Figure 4). Furthermore, biochemical clinical parameters were analyzed relative to the expression of both microRNAs. Conversely, the findings of the relative expression of miR-365 found to be positively correlated with fasting glucose, LDL and insulin levels. The findings of the relative expression of miR-375 found to be negatively correlated with HDL and insulin levels as in (Figure 2).

Table 1: Clinical description of the participants in the present study

Markers	**Control Group** **(n= 25)** (Mean, Standard deviation)	**Obese without T2D** **(n= 30)** (Mean, Standard deviation)	**Obese with T2D** **(n= 30)** (Mean, Standard deviation)
BMI (Kg/m^2)	21.27±2.475	37.1±4.495	45.54±6.786
Cholesterol	155±4.99	225±10.25	198±9.74
HDL	49.92±4.153	57.63±7.757	55.77±5.581
LDL	77.24±4.245	97.14±40.77	119.6±59.28
Triglycerides	86.2±14.16	126.8±46.85	173.7±52.23
Fasting Glucose (mg/dl)	89.4±10.54	86.73±8.469	132.7±8.674
Insulin	2.972±0.6248	8.463±3.786	6.247±3.898
HOMA-IR	0.6476±0.1437	1.862±0.974	2.039±1.223
Urea	22±3.44	22.9±3.144	23.87±3.06
Creatinine	0.858±0.1296	0.9007±0.1254	0.889±0.1135
ALT	23.6±3.175	23.53±3.014	21.8±3.662
AST	18.28±5.42	23.27±3.956	22.77±3.461

Table 2: Dunn's multiple comparisons test of Kruskal-wallis statistical model for different parameters in different groups

Dunn's multiple comparisons test	*p-values*		
	Control vs. Obese	Control vs. Diabetic	Obese vs. Diabetic
BMI (Kg/m^2)	*0.0001**	*0.0001**	*0.0015**
Cholesterol	*0.0014**	*0.0001**	*0.258*
HDL	*0.0001**	*0.001**	*0.8389*
LDL	*0.6645*	*0.1362*	*0.9999*
Triglycerides	*0.0023**	*0.0001**	*0.0029**
Fasting Glucose (mg/dl)	*0.9999*	*0.0001**	*0.0001**
Insulin	*0.0001**	*0.0011**	*0.0394**
HOMA-IR	*0.0001**	*0.0001**	*0.9999*
Urea	*0.8674*	*0.0852*	*0.7059*
Creatinine	*0.5027*	*0.9331*	*0.9999*
ALT	*0.9999*	*0.3762*	*0.1181*
AST	*0.9999*	*0.421*	*0.215*

**p-value is significant*

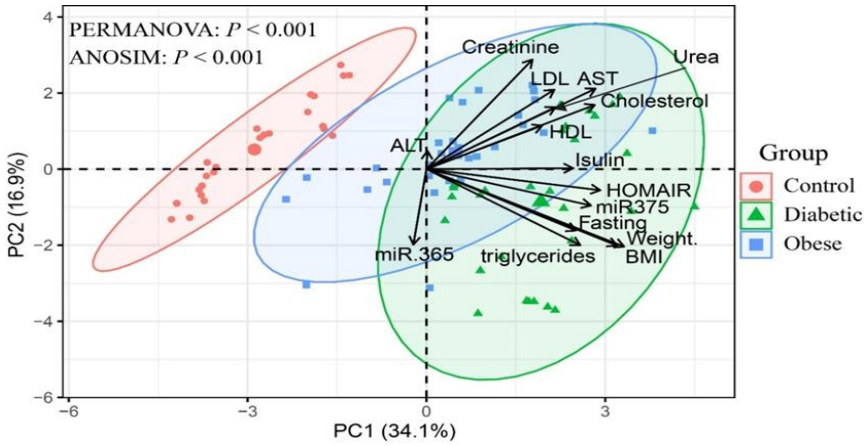

Figure 1: Principal component analysis describes the grouping pattern of the individuals in the present study.

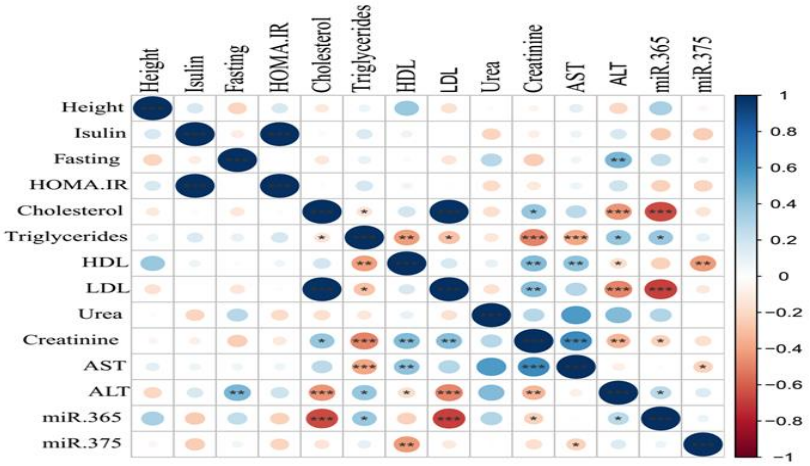

Figure 2: Correlations between different groups using Pearson matrix correlation.

The numbers within the cells represent the value of correlation.
The circles within cells display the significant correlations.

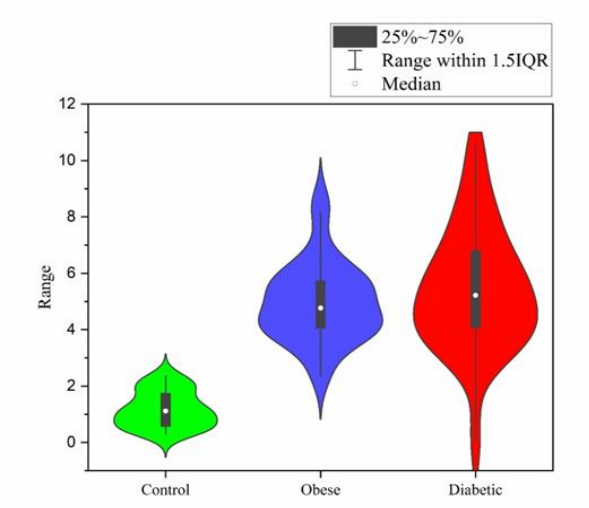

Figure 3: Relative expression of miR-365 in different groups

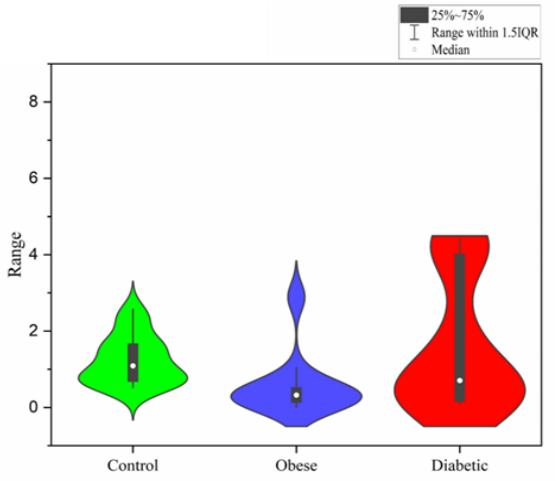

Figure 4: Relative expression of miR-375 in different groups

4. DISCUSSION

We investigated the expression of circulating levels of miR-365 and miR-375 and their relationship with biochemical clinical factors associated with obesity with or without T2D development in this study. However, the microRNAs were linked to clinical characteristics linked to obesity or T2D metabolic problems. Thus, we undertook a systematic investigation to determine the physiological stresses that may be impacting the relative expressions of miR-365 and miR-375. MiR-375 is required for cell survival [12], and its levels in the blood might be employed as a marker of cell death [14]. The current results found that the expression of miR-375 was considerably reduced in obese people with or without T2D, which is similar with a recent study found that miR-375 was substantialy raised among people with type-1 diabetes (T1D) [20]. In this regard, we discovered that miR-375 expression was adversely related to HOMA.IR, HDL, and insulin. These findings are similar with a recent publication that demonstrated miR-375, which is abundantly expressed in pancreatic B-cells, inversely controls insulin exocytosis and that miR-375 over expression reduced glucose-induced insulin production by down-regulating myotrophin [21]. We revealed that miR-365 was more expressed in obese people with or without T2D compared to controls, which is consistent with a previous study on mouse cells from interscapular

brown fat revealed that the brown fat-enriched miRNA cluster miR-193b-365 was a crucial regulator for the development of brown fat that showed a significant up-regulation [16].

In this regard, our findings are consistent with the findings of a single research that found miR-365 to be a key regulator of brown fat differentiation and to have a role in obesity [16]. We also discovered that miR-365 expression was inversely related to insulin, cholesterol, and low-density lipoprotein levels. It might demonstrate the management of brown fat fates via adipogenesis and myogenesis. Sun et al. conducted a comparative analysis of the epididymal WAT, skeletal muscle, and interscapular BAT using the genome-wide miRNA profile expression. They found that the WAT samples showed an up-regulation of miR-365 during adiposeness, and a down-regulation of up to 30% in the BAT of ob/ob mice (metabolic impairment) and concluded that these miRNA profiles can function as crucial regulators for brown fat differentiation, partly through inhibiting the production of myogenesis perilipin 5 (PLIN5), a protein that coats lipid droplets inside cells, especially in BAT, and by promoting mitochondrial function in brown adipocytes cell line. The elevated levels of islet PLIN5 during fasting facilitates the partitioning of fatty acids into lipid droplets, which are released following refeeding to improve postprandial insulin production in cAMP- and GPR40-dependent manners. Activated brown adipose tissue has the ability to ameliorate metabolic disorders like insulin resistance, dyslipidemia, and alterations in glucose homeostasis in addition to promoting negative energy balance [22, 23]. The selective uptake of fatty acids from triglyceride-rich lipoproteins into BAT is facilitated by BAT activation, which in turn accelerates the liver's clearance of the cholesterol-enriched remains. The functional hepatic apoE-LDLR clearance pathway is necessary for these effects, since BAT stimulation does not reduce hypercholesterolemia in Apoe−/− and Ldlr−/− mice [24]. Consequently, once the apoE-low-density lipoprotein receptor pathway is intact, BAT activation results in the production of lipoprotein remnants that are then eliminated by the liver. Via these pathways, BAT activation lowers cholesterol and plasma triglyceride levels and delayed the onset of diet-induced atherosclerosis [22].

5. CONCLUSION

The present study provides evidence of distinct expression profiles of miR-365 and miR-375 in obese individuals with and without T2D. Up-regulation of miR-365 and down-regulation of miR-375 suggest their involvement in the development and progression of these disorders. Further investigations are warranted to elucidate the precise molecular targets and signaling pathways affected by these miRNAs and to explore their therapeutic potential in managing obesity and T2D.

LIST OF ABBREVIATIONS

Type-1 diabetes (T1D); type-2 diabetes (T2D); MicroRNAs (miRNAs); body mass index (BMI); fasting blood sugar (FBS); Homeostatic Model Assessment (HOMA) IR; Quantitative real-time PCR (RT-qPCR); triglyceride (TG); total cholesterol (TC); high density lipoprotein (HDL) cholesterol; low density lipoprotein (LDL) cholesterol; aspartate aminotransferase (AST); alanine aminotransferase (ALT); principal component analysis (PCA); perilipin 5 (PLIN5).

COMPETING INTERESTS

No conflict of interest.

AUTHOR CONTRIBUTIONS

The authors all contributed equally to the work and read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data generated and/or examined during the present study are not publicly available due to an ongoing research endeavor but are available upon reasonable request from the corresponding author.

ETHICAL CONSIDERATIONS

This study was approved by the Ethics Committee of Beni-Suef University, FMBSUREC10062018, 10-6-2018 in accordance to relevant guidelines and regulations or declaration of Helsinki and a written informed consent was obtained from all subjects.

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