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Impact of Some miRNAs Expression on Induction of Obesity Related Diseases

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Abstract

Background: Obesity is a concerning health problem globally. Previous studies demonstrated the role of miR-15a and miRNA Let-7 in adipocyte development and in regulation of glucose metabolism.Blood samples of 65 obese patients (17 obese hypertensive patients, 24 obese diabetic patients, and 24 obese without complications) and 45 healthy volunteers were used as control.Fasting blood glucose, fasting insulin, lipid profile and HOMA-IR as well as miRNA-15a and miRNA Let-7 expression levels were assessed. **Results:** All tested biochemical parameters were significantly increased in all obese groups compared with the controls. The expression of miRNA 15a was significantly decreased in all obese groups in comparison with the controls especially the obese diabetic group, while the expression of miRNA Let-7 showed no significant difference between the studied groups with only a significant increase in obese hypertensive group. **Conclusion:** MiR15a level was reduced in the obese diabetic group, this finding highlights the role of miR15a in diabetes development. MiRNA Let-7 expression was decreased in diabetic obese patients while increased in hypertensive obese, suggesting its value in progression of diabetes and hypertension as obesity complications.

Keywords: Obesity; diabetes; hypertension; miRNA-15a; miRNA Let-7.

1. Introduction

Obesity is a complex condition marked by too much fat deposit that can result in the emergence of metabolic abnormalities. Thus it is considered as an important global health issue [1]. The links between metabolic diseases and obesity are deeply complicated. Though, dysregulation of molecules originating from adipose tissue could have a significant influence [2]. There is mounting evidence that miRNAs in circulation serve as paracrine and endocrine messengers, facilitating communication among tissues or cells. Many circulating miRNAs that regulate pathological and physiological processes in metabolic organ crosstalk have been identified in recent years [3].MicroRNA (miRNA) is a minute non-coding RNA that has undergone extensive conservation from worms to humans. It modulates a variety of metabolic processes dependent on its ability to bind to the three primary untranslated regions (3'-UTR) of the target mRNAs to inhibit translation or cause mRNA degradation to repress the corresponding target gene expression [4, 5]. Circulating miRNAs linked to adipose tissue or obesity could involve an array of non-invasive biomarkers that can be used to control obesity and related metabolic diseases [3]. Previous research has found that miR-15a/b expression levels change dramatically during adipogenesis [6]. MiR-15a has also been linked to the regulation of uncoupling protein-2 (UCP-2) and the promotion of insulin biosynthesis [7]. Another study found that miR-15acould suppress 3T3-L1 differentiation via the Delta-like 1 homolog (Dlk1) [8]. Furthermore, over-expression of miR-15b reduced cell proliferation while increasing intracellular triglyceride levels in QSG7701 cells [9]. These findings suggested that miRNA-15a may contribute to the differentiation of adipocytes. However, it is still unclear how miR-15a acts during adipocyte development. Recently, it was discovered that Let-7 controls the metabolism of glucose [10]. Clinically, let-7 has become one of the most significant regulators of metabolism, and a genome-wide association study (GWAS) found that let-7 target genes are linked to type 2 diabetes (T2D) [11]. Accordingly, this study's objective was to explore the clinical potential of two circulating miRNAs, miR-15a and Let-7, as possible biomarkers for predicting the possibility of developing metabolic diseases in the future and as treatment targets for obesity-related diabetes or hypertension.

2. Subjects and Methods

Study design and subjects

A total of 65 obese patients with BMI $\geq$ 30 kg/m^2 (17 obese with hypertension, 24 obese with diabetes and 24 obese without complication) who undergone bariatric surgery for obesity from surgery Unit-Kasr Al Aini Hospital, Cairo, Egypt were recruited in this study; their ages ranged between 25 and 60 years. Besides, 45 age- and sex matched healthy adult volunteers

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with no medical history of obesity or its complications were included as controls. A written informed consent was acquired from each participant before taking part in this research, under protocols approved by the Egyptian National Research Center's ethical committee (No. 19-162). Participants were excluded if one or more of the following exclusion criteria were met: liver disease, kidney disease and past history of steroid treatment.

Sampling

About 5 mL of peripheral venous blood were obtained after a 12-h fast from every individual. Blood was allowed to clot at room temperature (25 °C), centrifuged at 3000 g for 10 minutes, and then the sera were divided into two portions: one for biochemical analysis, and the other for adding to QIAZol in specially marked, sterile tubes for each individual subject, which were then stored at -80 °C until miRNA expression levels were determined.

Anthropometric measures

Body mass index (BMI) was determined as weight in kilograms (Kg) divided by height in meters squared (m^2).

Biochemical analysis

Fasting blood glucose (mg/dl): Blood glucose level was measured enzymatically by SPINREACT kit, Spain [12].

Fasting insulin (µlU/ml): Fasting insulin level measured by ELISA kit from Diagnostic Automation/Cortez Diagnostics, Inc (USA).

HOMA-IR (µlU.mol/l^2): The following equation, using the following formulas, HOMA-IR = [fasting insulin (µU/ml) × fasting plasma glucose (mg/dl)]/405 was used to calculate HOMA IR depending on the value of fasting glucose and insulin [13].

Lipid profile: Using a kit from Stanbio laboratory (USA), serum concentrations of total cholesterol (TC), triglycerides (TG), and HDL-c were calorimetrically determined. Using Friedewald's formula, LDL-c was calculated as LDL-c = TC - HDL-c - TG/5 [14].

MiRNAs expression assessinglevels assay

According to the manufacturer's instructions, total RNA was obtained using the miRNeasy Mini isolation kit (Cat. No. / ID: 217084) from QIAgen, Germany. The microRNA reverse transcription (RT-PCR One-Step) Kit (Cat # 12594100; Thermo Fisher; USA) and particular miRNA reverse transcription (RT) primers were used in accordance with the manufacturer's instructions to reverse-transcribe miR-15a and miR-Let 7. MiR-15a primers were (F): TAGCAGCACATAATGGTTTGTG; (R): GTGCAGGGTCCGAGGT[15] and miR-Let 7 primers were (F): CCAGCTGGGTGAGGTAGTAGGTTGT; (RT): CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATAC [16].RNA quantity and quality was measured using NanoDrop 2000c spectrophotometer® (Thermo Fisher Scientific Inc. USA). According to measurement of the A260/A280 ratios, all RNA samples are of adequate quality for qPCR analysis. One µL miRNA assays was mixed with 2 µL of RT products, 10 µL of SYBR green PCR master mix, and added nuclease-free water to obtain 20 µL as a final volume. The Step One Real-time PCR system (Thermo Fisher Scientific Inc. Waltham, MA, USA) was used for quantitative real-time analysis, all reactions were carried out under the following conditions: 95 °C for 10 min, then 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Target miRNA relative expression was normalised to U6. Using the equation $2^{-\Delta\Delta Ct}$, fold changes in candidate miRNA expression were estimated [17].

Statistical analysis

Analysis of data was implemented using the software SPSS (Statistical Package for the Social Sciences) version 20 (SPSS INC. Chicago, IL, USA). The quantitative variables were defined using their means and standard error. To compare groups, analysis of variance ANOVA test was used. Correlations between miRNAs expression levels and biochemical parameters were done by Spearman rank and Pearson correlation coefficients for skewed and normally distributed values, respectively. Significant expression levels were graphically represented by boxplot graphs. When the difference between the groups achieved $P< 0.05$, it was declared statistically significant, and when it reached $P< 0.01$, it was deemed extremely statistically significant.

3. Results

A total of 65 obese individual; out of them 17 cases were obese with hypertensive, 24 cases were obese and diabetic and 24 noncomplicated obese. Demographic data, clinical features and biochemical characters of all studied cases (65) and control individuals (45) were summarized in table 1. The results show no impact regarding age (P= 0.118) and sex (P= 0.275). However, comparison between different studied cases and control individuals regarding BMI mean shows a significant difference (P< 0.001). Also, biochemical characters including; fasting insulin, blood glucose, cholesterol, triglycerides, LDL, and HDL-cholesterol show a significant difference (P< 0.001 for all parameters) in different studied cases and control individuals. Regarding to the expression of circulating miRNAs; comparison between control group and all obese cases resulted in a significant difference in miRNA 15a (2.4 ± 0.23 vs. 1.2 ± 0.15 & P< 0.001) respectively (Fig. 1). However, circulating miRNA Let-7 expression shows no significant impact between control group and all obese cases (Fig. 2) (Table2). Similarly, comparison between control, obese, obese with hypertensive and diabetic obese groups regarding the expression of circulating miRNA 15a (2.4±0.2, 1.4±0.32, 1.3±0.2, 0.88±0.23, respectively) shows a significant impact (P< 0.001) (Fig. 3), also the expression of circulating miRNA Let-7 (1.5±0.1, 1.2±0.2, 2.5±0.4, 0.99±0.2, respectively) shows a significant difference (P= 0.001) (Fig. 4 and Table 3).

Coefficients of Person and Spearman correlation were tested between the expression of circulating miRNAs and different anthropometric and biochemical parameters in obese patients (Table 4). Regarding miRNA 15a expression, it shows a positive significant correlation with age (r= 0.296 & P= 0.017) and negative significant correlation with weight, BMI, total cholesterol and LDL-cholesterol (r= -0.310 & P=0.012, r= -0.297 & P= 0.016, r= -0.268 & P= 0.031, r= -0.296 & P= 0.017, respectively). On the other hand, miRNA Let-7 expression shows a positive significant correlation with age (r= 0.356 & P= 0.004) and a negative significant correlation with insulin level and HOMA IR (r= -0.344 & P= 0.005 & r= -0.331 & P= 0.007, respectively).

The linear regression model revealed that the biochemical features of obese individual, including fasting glucose level (P= 0.008), insulin level (P=0.018) and HOMA IR (P= 0.038) were independently associated with miRNA 15a expression (Table 5). Person correlation was tested between the expression of circulating miRNAs and different demographic and biochemical parameters in obese hypertensive patients (Table 6). The test indicated that there was a positive significant correlation between miRNA 15a expression and age, and triglycerides, also, a negative significant correlation between miRNA 15a expression and sex, insulin level HOMA IR, total cholesterol and LDL. Regarding miRNA Let-7 expression, there was a positive association with age, fasting glucose, and triglycerides, and a negative correlation with sex, insulin level, HOMA IR, total cholesterol and LDL.Coefficients of Person and Spearman correlation were tested between the expression of circulating miRNAs and different anthropometric and biochemical parameters in obese diabetic patients (Table 7). Regarding miRNA 15a expression, it shows a positive significant correlation with age and triglycerides. On the other hand, miRNA Let-7 expression shows a positive significant correlation with sex, BMI and triglycerides and a negative significant correlation with HDL.

Table 1: Anthropometric and clinical characteristics of study subjects

Variable	Controls (n=45)	Obese (n=24)	Obese Hypertensive (n=17)	Obese Diabetic (n=24)	*P* value
Demographic data					
Age (Years)	39.78±0.85	35.96±2	40.59±1.88	42.33±2.8	0.118
Sex (n %)					
Male	24 (53.3%)	8 (33.3%)	8 (41.1%)	8 (33.3%)	0.275
Female	21 (46.7%)	16 (66.7%)	9 (52.9%)	16 (66.7%)	
Anthropometric measurements					
Weight (Kg)	60.20±1.9	107.63±4	103.82±3.6	124.96±3.5	**<0.001**
Height (cm)	168.82±1.2	164.54±2.1	171.06±2.4	167.13±1.3	0.096
BMI (kg/m2)	21.09±0.37	37.9±0.9	35.9±1.7	44.6±1.4	**<0.001**
Biochemical characteristics					
Fasting blood glucose (mg/dl)	89.11±1.3	86.13±1.7	89.18±1.2	132.2±1.4	**<0.001**
Fasting insulin (µlU/ml)	3.1±0.1	7.8±0.7	5.3±0.9	5.8±0.91	**<0.001**
HOMA-IR (µlU.mol/l2)	0.68±0.02	1.7±0.2	1.1±0.19	1.9±0.28	**<0.001**
Triglycerides (mg/dl)	88.78±2	125.6±1.35	141±1.7	155.8±1	**<0.001**
Total cholesterol (mg/dl)	146.6±1.5	178.4±1.9	179.8±1.8	218.5±1.7	**<0.001**
HDL-cholesterol (mg/dl)	50.8±0.6	58.2±1.7	55.1±0.8	56.5±0.9	**<0.001**
LDL-cholesterol (mg/dl)	78.1±0.5	95±2.6	96.5±2.1	130.8±2.5	**<0.001**

Numeric variables are presented as mean ± SE or number (percentage)
P value for comparison between obese and control groups,
P value <0.05 are represented in bold font and considered as statistically significant,
HOMA-IR: homoeostasis model assessment-insulin resistance,
HDL: High density lipoprotein, LDL: Low density lipoprotein.

Table 2: The expression of circulating miRNAs (miR-15a &miR Let-7) in obese and control groups

MicroRNA	Control (n=45)	Obese (n=65)	P-value
miRNA 15a	2.4±0.23	1.2±0.15	**<0.001**
miRNA Let-7	1.55±0.13	1.47±0.15	0.727

Numeric variables are presented as mean ± SE.
P value for comparison between obese and control groups.
P value <0.05 are represented in bold font and considered as statistically significant.

Table 3: The expression of circulating miRNAs (miR-15a &miR Let-7) in obese, obese hypertensive, obese diabetic and control groups

MicroRNA	Control (n = 45)	Obese (n=24)	Obese Hypertensive (n=17)	Obese Diabetic (n=24)	P-value
miRNA 15a	2.4±0.2	1.4±0.32	1.3±0.2	0.88±0.23	**<0.001**
miRNA Let-7	1.5±0.1	1.2±0.2	2.5±0.4	0.99±0.2	**0.001**

Numeric variables are presented as mean ± SE.
P value for comparison between obese and control groups.
P value <0.05 are represented in bold font and considered as statistically significant.

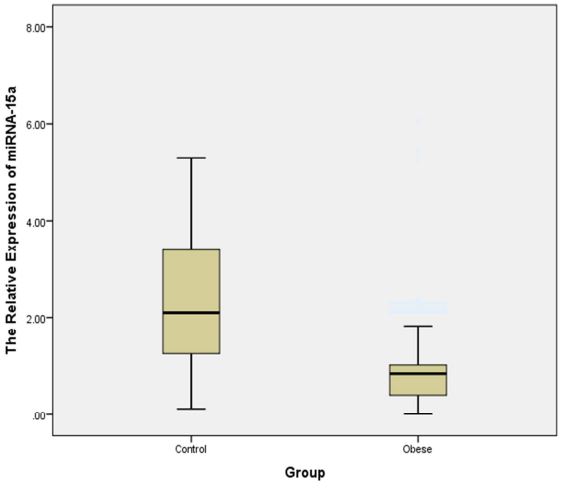

Figure 1: The relative miRNA-15a expression in obeseand control groups
Control group: Healthy subjects with normal weight.
Obese group: Obese patients.

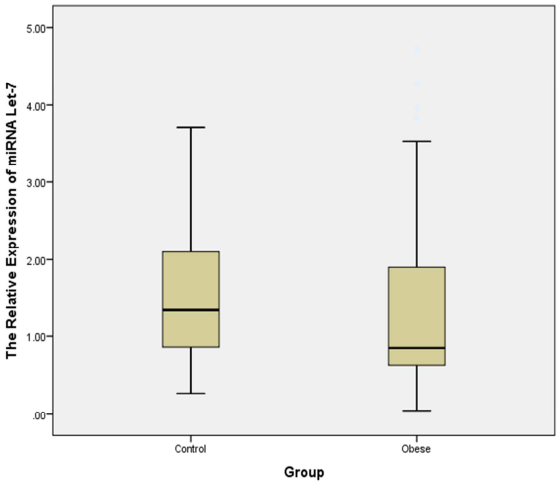

Figure 2: The relative miRNA Let-7 expression in obese and control groups
Control group: Healthy subjects with normal weight.
Obese group: Obese patients.

Table 4: Coefficients of Pearson and Spearman correlation between the expressions of miRNAs and different parameters in obese patients

		Age	Weight Kg	BMI Kg/m^2	Insulin	FG	HOMA IR	TC	TG	HDL	LDL
miRNA 15a	*r*	0.296*	-0.310*	-0.297*	-0.071	-0.175	-0.131	-0.268*	0.079	0.073	-0.296*
	P	**0.017**	**0.012**	**0.016**	0.573	0.164	0.299	**0.031**	0.531	0.561	**0.017**
miRNA Let-7	*r*	0.356**	-0.215	-0.212	-0.344**	-0.053	-0.331**	0.045	0.181	-0.239	-0.030
	P	**0.004**	0.086	0.089	**0.005**	0.675	**0.007**	0.724	0.149	0.055	0.813

(r) Pearson correlation for normally distributed values or Spearman correlation for skewed distribution values
* Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.
P value <0.05 are represented in bold font and considered as statistically significant.
FG: Fasting glucose HOMA-IR: Homoeostasis model assessment-insulin resistance
TC: Total cholesterol TG: Triglycerides HDL: High density lipoprotein LDL: Low density lipoprotein

Table 5: Linear regression analysis showing variables independently associated with miRNA-15a expression in obese patients

	Unstandardized Coefficients		Standardized Coefficients			95% Confidence Interval for B	
	B	Std. Error	Beta	t	Significant	Lower Bound	Upper Bound
Fasting Glucose	-0.035	0.013	-0.668	-2.734	**0.008**	-0.061	-0.009
Insulin	-0.514	0.212	-1.670	-2.429	**0.018**	-0.937	-0.091
HOMA IR	1.640	0.773	1.465	2.122	**0.038**	0.095	3.186

P value <0.05 are represented in bold font and considered as statistically significant.
HOMA-IR: Homoeostasis model assessment-insulin resistance.

Table 6: Correlations of miRNA-15a and miRNA Let-7 with different parameters in obese hypertensive patients

		Age	Sex	Insulin	FG	HOMA IR	TC	TG	HDL	LDL
miRNA 15a	Pearson Correlation	0.920**	-0.733**	-0.661**	0.196	-0.590*	-0.652**	0.518*	0.022	-0.672**
	Significant	**0.000**	**0.001**	**0.004**	0.451	**0.013**	**0.005**	**0.033**	0.932	**0.003**
miRNA Let-7	Pearson Correlation	0.821**	-0.771**	-0.707**	0.498*	-0.603*	-0.593*	0.612**	-0.022	-0.641**
	Significant	**0.000**	**0.000**	**0.002**	**0.042**	**0.010**	**0.012**	**0.009**	0.934	**0.006**

* Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.
P value <0.05 are represented in bold font and considered as statistically significant.
FG: Fasting glucose HOMA-IR: Homoeostasis model assessment-insulin resistance
TC: Total cholesterol TG: Triglycerides HDL: High density lipoprotein LDL: Low density lipoprotein

Table 7: Coefficients of Pearson and Spearman correlations between the expressions of miRNAs and different parameters in obese diabetic patients

		Age	Sex	BMI Kg/m^2	FG	Insulin	HOMA IR	TC	TG	LDL	HDL
miRNA 15a	*r*	0.454*	0.230	0.302	-0.158	-0.056	-0.069	-0.113	0.777**	-0.173	-0.030
	P	**0.026**	0.280	0.151	0.462	0.795	0.750	0.600	**0.000**	0.419	0.889
miRNA Let-7	*r*	0.134	0.703**	0.600**	0.163	-0.154	-0.157	-0.202	0.609**	-0.221	-0.617**
	P	0.531	**0.000**	**0.002**	0.447	0.473	0.463	0.343	**0.002**	0.300	**0.001**

(r) Pearson correlation for normally distributed values or Spearman correlation for skewed distribution values
* Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.
P value <0.05 are represented in bold font and considered as statistically significant.
FG: Fasting glucose HOMA-IR: Homoeostasis model assessment-insulin resistance
TC: Total cholesterol TG: Triglycerides HDL: High density lipoprotein LDL: Low density lipoprotein

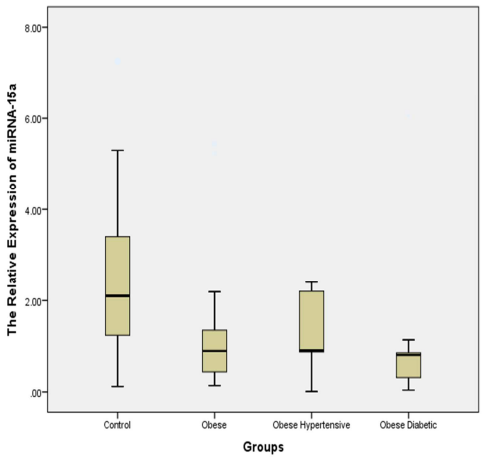

Figure 3: The relative miRNA-15a expression in obese, obese hypertensive, obese diabetic and control groups
Control group: Healthy subjects with normal weight.
Obese group: Obese patients without complications (Without hypertension or type two diabetes mellitus).
Obese Hypertensive group: Obese patients with hypertension as obesity complication.
Obese Diabetic group: Obese patients with type two diabetes mellitus as obesity complication.

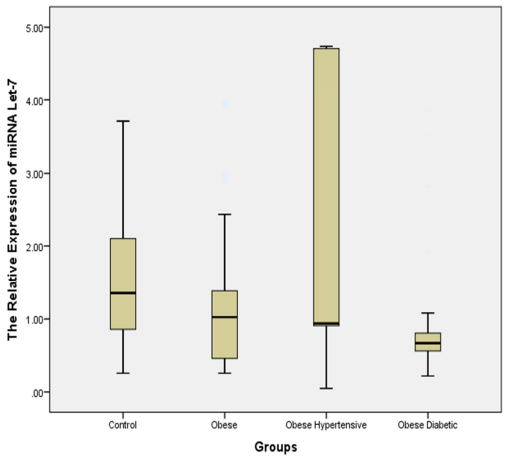

Figure 4: The relative miRNA Let-7 expression in obese, obese hypertensive, obese diabetic and control groups
Control group: Healthy subjects with normal weight.
Obese group: Obese patients without hypertension or type two diabetes mellitus.
Obese Hypertensive group: Obese patients with hypertension.
Obese Diabetic group: Obese patients with type two diabetes mellitus.

4. Discussion

The identification of circulating miRNAs as biomarkers for obesity could provide a further simple approach to assess the level of obesity and manage weight-loss therapy. Notably, future research into the function of circulating miRNAs in metabolic

diseases linked to obesity may lead to their utilization as risk factors for disease prognosis [3, 18]. Numerous particular miRNAs are known to have differential expression in obese patients as compared to normal individuals, and they may be used as biomarker prospects [3]. Although previous research has shown that miR-15a is crucial for several physiological processes, its significance in adipogenesis is yet uncertain. In order to clarify this, we evaluated miR-15a expression levels, and found that its expression levels decreased in obese patients compared with healthy controls. This finding is in agreement with previous study demonstrated that miR-15a is positive regulators during adipogenesis in pre-adipocyte. After day 4, miRNA-15a levels started to fall. It was higher throughout the early stages of adipogenesis [19, 20], which also matched the findings of a prior study using 3T3-L1 cells [8]. These findings suggested that miR-15a might be essential during the initial stages of adipogenesis. Additionally, pre-adipocytes with overexpressed miR-15a facilitated adipocyte differentiation and lipid accumulation. In the same line with our results, Ortega et al. found that morbidly obese patients show a notable reduction in circulating miR-15a [21].In our study, the expression levels of miR15a more decreased in obese patients with diabetic. This could be explained as T2DM is a complex disease characterized by pancreatic islet dysfunction. Remarkably, the transcription factor neurogenin3 (Ngn3), is an early marker of pancreatic islet cells and plays a significant role in the development of the endocrine lineages in mice [22], interferes with miR-15a expression [23]. These findings indicated that miR-15a might be crucial to the etiology of diabetes. The present study found that the circulating miRNA Let-7 expression level was not significantly differed between obese patients and control subjects, however the miRNA Let-7 expression level was statistically significantly differed in obese group with hypertension and diabetes compared to control group. Our finding is in the same line with a study revealed that let-7 directly influences glucose metabolism and insulin resistance in mice by acting on sites related to the insulin/IGF-1R pathway [11]. Reduced expression of let-7 prevented animals in let-7 knockout mice from becoming obese despite diet-induced insulin resistance, suggesting that let-7 may be a promising therapeutic target for diabetes [24]. More so Li et al. found let-7 is enhanced in hypertension [25]. A decrease in plasmatic levels of numerous members of the let-7 family of miRNA is connected to obesity and T2DM [26]. In addition, Arner et al. and Catanzaro et al. reported that obese participants expressed less let-7 than lean subjects did [27, 18]. Therefore, a decline in the expression of the let-7 family of miRNA in T2DM and obesity may result in an increase in pro-inflammatory cytokines that encourage insulin resistance [28].

5. Conclusion

MiRNAs have an essential role in the regulation of physiological and pathological processes in metabolic organs. This study investigated fasting blood glucose, fasting insulin, triglycerides, lipid profile, and HOMA-IR which were significantly increased in all obese groups compared with the controls. The expression patterns of miR-15a were significantly decreased in all obese groups in comparison with the healthy controls, especially the obese diabetic group. While the expression patterns of miR Let-7 displayed no significant difference among the obesity and control groups, with a significant increase in obese hypertensive patients and decreased with diabetic groups. So the miRNAs could be considered as a valuable keystone for upcoming studies on the biomarker ability of these miRNAs for T2D and hypertension.While more studies are needed to further explain these miRNAs roles.

Competing Interests

No conflict of interest.

Author Contributions

All of the authors have agreed to be fully responsible for the submitted manuscript's content and have given their approval for submission.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author.

Ethical Considerations

This study was approved by the Ethics Committee of National Research Centre (No. 19-162) and a written informed consent was obtained from all subjects. All methods were carried out in accordance with relevant guidelines and regulations and all experimental protocols were approved by National Research Centre.

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