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Original Article

Circulating miR-135b as a Biomarker of Obesity-Related Insulin Resistance and Dyslipidemia in Children and Adolescents

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ABSTRACT

Objectives: Childhood obesity is a global health concern associated with long-term metabolic complications. MicroRNA-135b (miR-135b) has been implicated in regulating adipogenesis, glucose metabolism, and insulin signaling, partly by directly targeting SIRT1, a key metabolic regulator that plays a crucial role in energy homeostasis and inflammation. However, the role of miR-135b in pediatric obesity remains unclear. This study aimed to investigate circulating miR-135b level and its relationship with SIRT1 expression, lipid profile, and glycemic parameters in children and adolescents with obesity.

Methods: A total of 67 participants (36 obese and 31 normal-weight controls) aged 8–16 years were enrolled. Anthropometric measurements and biochemical analyses were performed. miR-135b and SIRT1 expression levels were measured using quantitative real-time PCR. Insulin resistance was assessed using HOMA-IR, and metabolic syndrome was diagnosed based on International Diabetes Federation criteria.

Results: miR-135b expression was significantly elevated in the obesity group compared to controls and was highest among participants with insulin resistance and metabolic syndrome. Elevated miR-135b correlated positively with BMI z-score, insulin levels, HOMA-IR, total cholesterol, triglycerides, and LDL-C, while showing no significant correlation with HDL-C. In contrast, SIRT1 expression was significantly decreased in obese individuals ($p = 0.0026$) and inversely correlated with miR-135b levels.

Conclusion: Elevated miR-135b and reduced SIRT1 expression are associated with obesity-related metabolic disturbances in children and adolescents. These findings suggest that the miR-135b/SIRT1 axis may play a pivotal role in the development of insulin resistance and dyslipidemia, highlighting miR-135b as a potential biomarker and therapeutic target for early intervention in pediatric obesity.

Keywords: miR-135b; Obesity; Sirtuin 1; Metabolic syndrome; Insulin resistance

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Introduction

Childhood obesity has emerged as a major global health challenge, with its prevalence rising dramatically over the past four decades. Current estimates indicate that approximately 18.5% of children and adolescents aged 2 to 19 years are affected, amounting to around 13.7 million individuals worldwide (1). This upward trend is concerning, as obesity during childhood often tracks into adulthood, with about 75% of affected children remaining obese later in life, significantly increasing their risk of cardiometabolic disorders and premature mortality (1, 2). The complications of childhood obesity are extensive and include insulin resistance, dyslipidemia, hypertension, and metabolic dysfunction-associated steatotic liver disease (3). These conditions collectively contribute to the development of metabolic syndrome (MetS), a cluster of cardiometabolic abnormalities including central obesity, hyperglycemia, hypertension, and dyslipidemia (4). Given its high prevalence and long-term health implications, childhood obesity represents a critical target for early intervention. Preventing obesity in early life can reduce the global burden of cardiovascular disease and other noncommunicable diseases, underscoring the urgent need for novel strategies focused on preventive actions (2).

Sirtuin 1 (SIRT1), the mammalian ortholog of yeast Sir2, is a highly conserved NAD-dependent protein deacetylase that functions as a key metabolic sensor, linking environmental nutrient signals to the regulation of metabolic homeostasis. Studies have shown that artificial overexpression of SIRT1 suppresses inflammatory responses, whereas its deletion in hepatocytes leads to increased local inflammation (5, 6). In differentiated adipose cells, SIRT1 upregulation decreases fat storage and enhances lipolysis, indicating its pivotal role in adipocyte function (7, 8). Furthermore, SIRT1 has been implicated in regulating adipocyte formation and activity in response to dietary restriction (9). Consistently, transgenic mice overexpressing SIRT1 exhibit a leaner phenotype, higher metabolic activity, and lower serum levels of cholesterol, adipokines, insulin, and glucose compared to controls (5, 10, 11).

Adipocyte stress and hypertrophy trigger the release of adipocyte-derived exosomes carrying bioactive molecules, including microRNAs (miRNAs). These miRNAs serve as critical mediators of intercellular communication, playing essential roles in regulating metabolic responses (12). Each miRNA possesses a distinct signature that influences the metabolic activity of target cells, often by interacting with multiple messenger RNAs (mRNAs), whose coordinated or opposing effects shape cellular functions (13). During obesity and its related comorbidities, several miRNAs become dysregulated in various tissues, contributing to pathological changes (14-17). Processes such as adipocyte differentiation and lipid droplet formation, macrophage recruitment and inflammation, lipolysis, lipotoxicity, and insulin resistance are tightly regulated by miRNAs (18-21). As key epigenetic regulators, miRNAs not only modulate gene expression but also represent valuable biomarkers for identifying novel gene transcripts and potential therapeutic targets.

SIRT1 can be directly targeted and inhibited by various microRNAs, leading to altered cellular functions and disease progression (22-24). miR-135 directly targets and inhibits SIRT1, and through this mechanism, it contributes to diverse pathological processes, such as promoting trophoblast proliferation and gestational diabetes development (25), inducing renal fibrosis in diabetic nephropathy via activation of the TGF-β/Smad3 pathway (26), and impairing DNA damage repair during cellular reprogramming (27). Given the critical role of miRNAs in regulating key metabolic pathways and their interactions with SIRT1, the aim of the present study was to measure circulating miR-135 levels in children and adolescents with obesity and compare them with those of healthy controls. In addition, we investigated the relationship between miR-135 levels and lipid profile parameters as well as glycemic indices to better understand its potential role in obesity-related metabolic disturbances.

Materials and methods

Subjects and Study Design

A total of 67 participants, aged 8 to 16 years, were recruited for this study, consisting of 36 children and adolescents with obesity and 31 age- and sex-matched healthy controls. All individuals underwent a detailed medical history review and a comprehensive physical examination before enrolment.

Anthropometric measurements included weight, height, waist circumference (WC), hip circumference (HC), and calculation of waist-to-hip ratio (WHR). Blood pressure was measured in a seated position, and both systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were recorded. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2), and corresponding BMI z-scores and percentiles were determined based on sex- and age-specific reference standards. Participants with a BMI at or above the 95th percentile were classified as obese. In contrast, those with BMI values between the 5th and 85th percentiles were considered to have a normal weight and were included in the control group. Children with any current or past chronic illnesses, or those taking medications that could influence metabolic parameters, were excluded from participation.

Assessment of Insulin Resistance and Metabolic Syndrome

Insulin resistance was evaluated using the

homeostatic model assessment of insulin resistance (HOMA-IR), calculated by the formula: HOMA-IR = (fasting insulin [μIU/mL] × fasting glucose [mg/dL]) / 405.

A HOMA-IR value greater than 3.16 was considered indicative of insulin resistance (28).

The diagnosis of metabolic syndrome (MetS) was established according to the International Diabetes Federation (IDF) criteria for children and adolescents. This required the presence of abdominal obesity, defined as a WC above the 90th percentile for age and sex, along with two or more additional risk factors, such as elevated fasting glucose, high triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C), or increased SBP/DBP.

Ethical Considerations

Written informed consent was obtained from all participants and/or their parents or guardians before inclusion in the study. The study protocol was reviewed and approved by the Ethics Committee of Iran University of Medical Sciences (ethics code IR.IUMS.FMD.REC.1399.268).

Sample Collection and Biochemical Analyses

After an overnight fasting period, venous blood samples were collected from all participants. Plasma was carefully separated into RNase-free tubes while kept at 4°C to preserve RNA integrity and then stored at –80°C until further processing for miRNA analysis. The remaining serum fraction was utilized for the measurement of biochemical parameters. Peripheral blood mononuclear cells (PBMCs) were separated from the blood samples using Ficoll-Hypaque (Lympholyte-H; Cedarlane Laboratories, Ontario, Canada) density gradient.

Serum levels of fasting plasma glucose (FPG), TG, total cholesterol (TC), HDL-C, and low-density lipoprotein cholesterol (LDL-C) were quantified using enzymatic colorimetric assays with commercially available kits (Parsazmoon, Iran). Insulin concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits obtained from Monobind (Iran).

MicroRNA Extraction and cDNA Synthesis

Plasma and PBMC RNA were extracted using the Favorgen MicroRNA and total RNA extraction Kits, separately (Yekta Tajhiz, Iran). This method involves a lysis step for the disintegration of exosomes, followed by phenol/chloroform separation and a column purification step. To ensure consistency and monitor extraction efficiency and technical reproducibility, an equal quantity of the cel-miR-39-1 (UCACCGGGUGUAAAUCAGCUUG) was added to each sample before the isolation procedure.

For complementary DNA (cDNA) synthesis, poly (A) polymerase (New England Biolabs, USA) was first used to attach a poly (A) tail to the isolated miRNAs, facilitating reverse transcription. Subsequently, cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). A specialized primer containing both a sequence complementary to the poly(A) tail and an adapter sequence was employed to enable downstream amplification (Table 1). SIRT1 gene expression was measured in PBMC-derived RNA using qRT-PCR.

Quantification of miRNA by Real-Time PCR

Quantitative real-time PCR (qRT-PCR) was carried out to determine plasma miRNA expression levels. Reactions were set up using the SYBR Green Master Mix (Ampliqon, Denmark) in combination with both miRNA-specific primers and a universal reverse primer (Table 1). Amplification was performed on an Applied Biosystems StepOne Real-Time PCR System with the following cycling conditions: an initial denaturation step at 95°C for 15 seconds, followed by 40 cycles consisting of denaturation at 95°C for 3 seconds and combined annealing/extension at 60°C for 30 seconds. To ensure accurate quantification, miR-16 was selected as the internal reference for normalization. In addition, cel-miR-39 was used to assess the Spike-In control, which had been added prior to RNA isolation, to verify the efficiency and reproducibility of RNA extraction. A standard curve ranging from 1×10^3 *to* 1×10^6 *copies* of cel-miR-39 cDNA was prepared separately from plasma samples and the RNA extraction process, allowing estimation of recovery rates for the Spike-In control.

Table 1. Sequences of primers

Primers	Sequence
hsa-miR-135b-5p forward	5′- TATGGCTTTTCATTCCTATGTG-3′
hsa-miR-16 forward	5′- GGGTAGCAGCACGTAAATATTGG-3′
Cel-mir-39 forward	5′-TCACCGGGTGTAAATCAGCTTG-3′
Universal reverse	5′- GCGAGCACAGAATTAATACGACTC-3′
Adaptor	5′- GCGAGCACAGAATTAATACGACTCACTATAGGTTTTTTTTTTTTAG -3′
SIRT1 forward	5′-TGCGGGAATCCAAAGGATAA-3’
SIRT1 reverse	5′-CAGGCAAGATGCTGTTGCA-3’
Beta-actin forward	5′-GCAAGCAGGAGTATGACGAG-3’
Beta-actin reverse	5′-CAAATAAAGCCATGCCAATC -3’

At the end of each PCR run, a melt curve analysis was performed to confirm the specificity of amplification. The relative expression levels of target miRNAs were calculated using the $2^{-\Delta Ct}$ method, defined as: ΔCt = Ct (target miRNA) – Ct (normalizer)

Statistical Analysis

Statistical evaluation was performed using MedCalc software (version 18.2.1, MedCalc Software Ltd., Belgium). Data normality was assessed using the Kolmogorov–Smirnov test. Comparisons between groups were carried out using the independent samples t-test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. A p-value < .5 was considered statistically significant.

Results

Baseline characteristics

The baseline demographic and clinical characteristics of the participants are summarized in Table 2. There were no significant differences between the obesity and control groups regarding age or gender distribution. Obesity parameters, including BMI, BMI z-score, WC, HC, and the WHR were significantly higher in the obesity group. However, there were no significant differences between the SBP and DBP of individuals with obesity compared to their normal-weight counterparts.

As shown in Table 3, although there was no significant difference in fasting plasma glucose (FPG) level in the two groups, serum insulin and HOMA-IR levels were significantly increased in the obesity group. A significant alteration in lipid profile was also observed between the two groups, with subjects in the obesity group showing less favorable lipid parameters. Within the obesity group, 36.1% of subjects met the diagnostic criteria for metabolic syndrome (MetS), and 72% were classified as insulin resistant (IR).

miRNA Expression Analysis

Results showed that there was a significantly higher level of miR-135b in subjects with obesity compared to control subjects (Figure 1A). Similarly, when

Table 2. Demographic characteristics of the study population.

	Control	Obesity	P value
Female/male	20/11	16/20	0.4593
Age (Years)	11.41 ± 2.2	10.98 ± 2.7	0.4914
BMI	17.99 ± 1.8	28.17 ± 5.7	<0.0001
BMI Z-score	0.240 (-0.9 - 0.6)	2.15 (1.8 - 2.3)	<0.0001
Height SDS	-0.50 (-1.6 - 0.1)	-0.005 (-0.5 - 0.8)	0.0008
Weight SDS	-0.48 (-1.4 - 0.2)	1.99 (1.4 - 2.4)	<0.0001
HC (cm)	78.0 ± 8.5	97.25 ± 13.6	<0.0001
WC (cm)	60.96 ± 6.5	84.33 ± 13.55	<0.0001
WC/HC	0.78 (0.7 - 0.8)	0.85 (0.8 - 0.9)	< 0.0001
SBP (mmHg)	100.0 (90 - 100)	100.8 (90 - 110)	0.1833
DBP (mmHg)	68.3 (60 - 70)	72.2 (60 - 80)	0.2913

Data is represented as mean ± SD for parametric and median (interquartile range) for non-parametric variables. BMI: Body mass index, SDS: standard deviation score, HC: hip circumference, WC: waist circumference, WC/HC: waist to hip circumference ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 3. Biochemical parameters of the study population.

	Control	Obesity	P value
FPG (mg/dL)	90.25 ± 5.6	92.25 ± 6.4	0.1860
Insulin (μU/mL)	4.80 (4.0 - 6.7)	19.90 (12.6 - 26.8)	<0.0001
HOMA-IR	0.98 (0.9 - 1.5)	4.37 (2.8 - 6.0)	<0.0001
Cholesterol (mg/dL)	150.0 ± 19.6	171.77 ± 25.6	0.0003
Triglycerides (mg/dL)	69.00 (52.5 - 82.0)	111.0 (72.5 - 162.0)	0.0001
LDL-C (mg/dL)	76.81 ± 13.9	91.11 ± 17.7	0.0006
HDL (mg/dL)	54.41 ± 7.2	48.27 ± 9.3	0.0041

Data is represented as mean ± SD for parametric and median (interquartile range) for non-parametric variables. FPG: fasting plasma glucose, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol.

participants with obesity were stratified based on the presence or absence of insulin resistance, a significant elevation was observed in the level of miR-135b in serum of those with insulin resistance (Figure 1B). A notable increase in serum miR-135b level was also detected in individuals with metabolic syndrome (MetS) compared to the control group ($p = 0.001$), as illustrated in Figure 1C. SIRT1 expression levels were investigated in the participants and compared between the two groups. The results revealed a significantly lower expression of SIRT1 in PBMCs of the obesity group compared with the normal-weight individuals (Figure 1D).

Correlation Analysis

The correlation analysis between circulating miR-135b level and various metabolic parameters revealed several significant associations (Table 4). miR-135b showed a strong positive correlation with BMI Z-score. Additionally, significant positive correlations were observed between miR-135b and insulin levels as well as HOMA-IR. Among the lipid profile parameters, miR-135b correlated positively with total cholesterol, triglycerides, and LDL-C, while no significant correlation was found with HDL-C. These findings imply that elevated miR-135b levels are associated with a more adverse lipid profile and greater metabolic dysfunction. When the correlations were analyzed in the obesity group separately, the correlation of miR-135b with metabolic parameters did not remain significant.

Since SIRT1 is a target of miR-135b, the correlation between miR-135b and SIRT1 gene expression level was analyzed and the results showed that there was a significant correlation between these two parameters (Figure 2A). Additionally, when this correlation was investigated in the obesity group individually, the correlation still remained significant (Figure 2B).

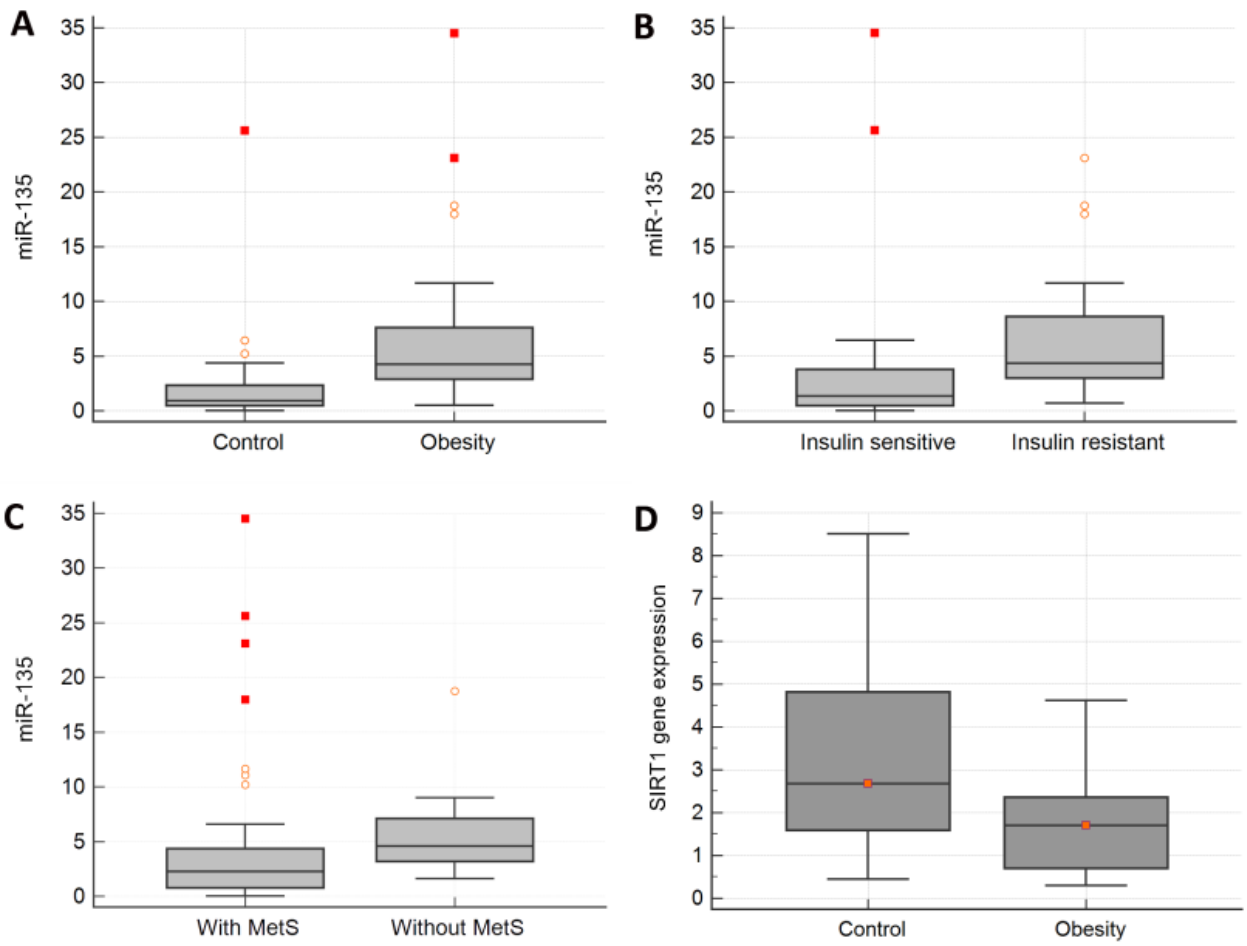

Figure 1. Comparison of miR-135b and SIRT1 levels in different groups, A: subjects with obesity and normal-weight control ($P < 0.0001$), B: obesity group divided based on the presence of insulin resistance ($P = 0.0001$), C: obesity group divided based on the diagnosis of metabolic syndrome (MetS) ($P = 0.0170$) and D: comparison of SIRT1 gene expression levels between the control and obesity groups ($P = 0.0026$).

Table 4. Correlation of miR-135b with metabolic parameters.

	r	P value
BMI Z-score	0.467	0.0001
WH/HC	0.1684	0.1732
FBS (mg/dL)	0.057	0.6473
Insulin (μU/mL)	0.497	<0.0001
HOMA-IR	0.494	<0.0001
Cholesterol (mg/dL)	0.396	0.0009
Triglycerides (mg/dL)	0.297	0.0148
HDL-C	-0.140	0.2578
LDL-C	0.342	0.0046

FBS: fasting blood sugar, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol.

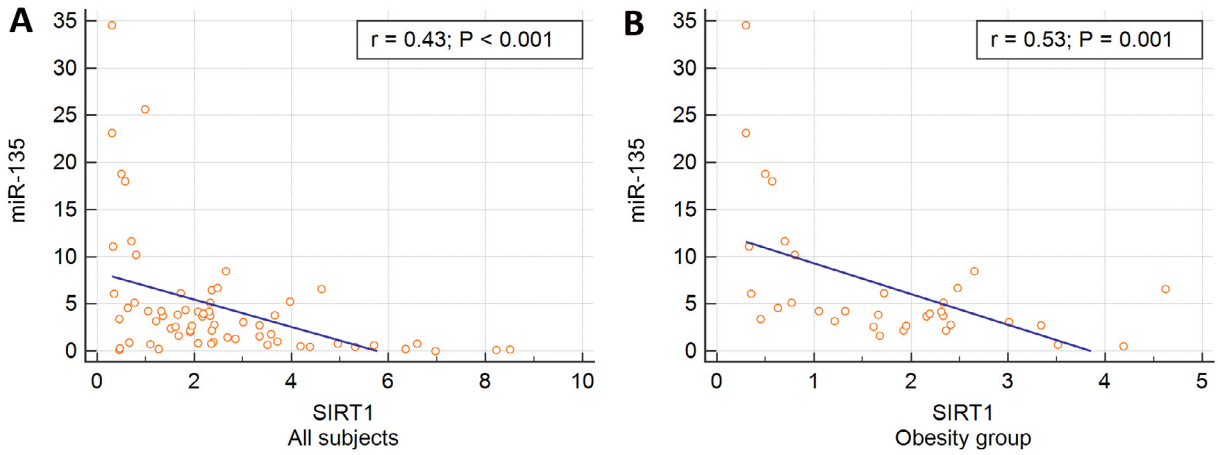

Figure 2. The correlation between miR-135b and SIRT1 in all subjects (A), and the obesity group separately (B).

Discussion

Here, we found higher expression of miR-135b in serum of subjects with obesity and a significant correlation between miR-135b and BMI z score in the studied population, indicating that higher levels of miR-135b are linked to increased adiposity. Previous studies have shown overexpression of miR-135b in adipocytes compared to human adipose-derived mesenchymal stem cells, and that upregulation of miR-135a-5p can promote proliferation and adipogenesis of these stem cells, indicating that miR-135a-5p plays an active role in adipogenesis (29). However, other findings demonstrate that miR-135a-5p suppresses the differentiation and adipogenesis of preadipocytes via the activation of Wnt/β-catenin signaling by directly targeting Apc (30). These controversial findings suggest that increased miR-135 in subjects with obesity may be a consequence, rather than a cause, of adiposity.

We observed higher expression of miR-135b in individuals with insulin resistance and a significant correlation between miR-135b and insulin levels, as well as HOMA-IR, suggesting a potential role of miR-135b in insulin resistance. Consistently, miR-135b has been introduced as a microRNA that takes part in the regulation of glucose uptake, insulin signaling pathway, and mitochondrial biogenesis in skeletal muscle (31). Several studies support the role of miR-135b in glycemic homeostasis. Studies have shown that miR-135a is up-regulated in the diabetic gastrocnemius skeletal muscle, and it inhibits insulin signaling and glucose uptake by targeting IRS2 (32, 33). Monfared et al. reported a significant elevation of miR-135a in diabetes and prediabetes patients. Additionally, they demonstrated a positive correlation between miR-135a and HbA1c levels in both prediabetes and diabetes status, thereby introducing this miRNA as a potential assistant marker for HbA1c in detecting type 2 diabetes. Insulin resistance is a key contributor to MetS, and elevated miR-135 levels in patients with MetS further support a role for miR-135 in the development of insulin resistance.

Multiple studies have demonstrated that various miRNAs regulate SIRT1 expression, thereby contributing to the development and progression of different diseases. For example, miR-155-5p was shown to directly target SIRT1 in cardiomyocytes, where its overexpression promoted pyroptosis via activation of the NLRP3 inflammasome, while inhibition of miR-155-5p protected against hypoxia/reoxygenation-induced myocardial injury (34). Similarly, miR-200a-3p was found to be elevated in the kidneys of patients with hypertension and diabetes, leading to downregulation of SIRT1 and increased tubular cell injury, while inhibition of miR-200a-3p restored SIRT1 expression and mitigated renal damage (35).

In the liver, several miRNAs have been implicated in lipid metabolism disorders. miR-23b directly binds to the 3′-UTR of SIRT1 mRNA, decreasing its expression and activity, thereby enhancing lipid accumulation in hepatocytes and potentially contributing to non-alcoholic fatty liver disease (23). Likewise, miR-141 was reported to suppress SIRT1 and, in turn, decrease AMPK phosphorylation in hepatocytes, thereby promoting hepatic steatosis and insulin resistance (22). Additional evidence shows that miR-93, by repressing SIRT1, promotes metabolic dysfunction-associated steatotic liver disease and accelerates its progression (36). Furthermore, miR-22 has been linked to neuroinflammation by modulating the miR-22-3p/SIRT1 pathway, influencing processes such as oxidative stress and neuronal apoptosis (37). Collectively, these findings highlight that dysregulation of SIRT1 by specific miRNAs constitutes a common pathogenic mechanism underlying diverse diseases, including cardiovascular, renal, metabolic, and neurodegenerative disorders.

miR-135 is among the miRNAs that target SIRT1 across different biological systems and diseases. In cellular models, miR-135a has been shown to negatively regulate SIRT1 expression, leading to decreased reprogramming efficiency and impaired DNA damage repair during the generation of induced pluripotent stem cells (27). In the context of gestational diabetes mellitus (GDM), placenta-derived exosomal miR-135a-5p was found to promote trophoblast cell proliferation, invasion, and migration by suppressing SIRT1 and activating the PI3K/AKT signaling pathway (25). Similarly, in diabetic nephropathy, miR-135a-5p was significantly upregulated, and its knockdown alleviated TGF-β1-induced renal fibrosis by targeting SIRT1 and inactivating Smad3 signaling (26). Moreover, in cardiovascular disease, miR-135a was reported to enhance atrial fibrosis through modulation of mitochondrial oxidative respiratory function by inhibiting SIRT1 (38). Collectively, these findings suggest that miR-135 serves as a crucial regulator of SIRT1 in various physiological and pathological processes, linking it to fibrosis, metabolic disorders, and cellular reprogramming.

In summary, in this study, we demonstrated that miR-135b expression is markedly elevated in children and adolescents with obesity, particularly among those with insulin resistance and metabolic syndrome. Elevated miR-135b levels were positively associated with adverse lipid profiles and markers of glycemic dysfunction, suggesting its potential role as a biomarker of obesity-related metabolic disturbances. In contrast, SIRT1 expression was significantly reduced in the obesity group and inversely correlated with miR-135b, supporting the notion that miR-135b may negatively regulate SIRT1 and contribute to the development of metabolic abnormalities. These findings highlight the potential role of miR-135b/SIRT1 signaling in the pathogenesis of pediatric obesity and its associated complications. Further longitudinal studies are needed to confirm the causal relationship between miR-135b and SIRT1 and to explore whether modulation of this pathway could serve as a therapeutic target for early intervention in obesity and metabolic syndrome.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics statement

This research was approved by the Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.FMD.REC.1399.268) and followed all ethical guidelines in accordance with the Declaration of Helsinki.

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