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Exploring serum miR-33b as a novel diagnostic marker for hypercholesterolemia and obesity: insights from a pilot case-control study

Yaser Masoumi-Ardakani[1], Mostafa Eghbalian[2], Hossein Fallah[3], Alireza Jafari[4] and Beydolah Shahouzehi[5*]

Abstract

Obesity and atherosclerosis are significant metabolic diseases characterized by disrupted lipid metabolism. MicroRNAs (miRNAs) are small, conserved, non-coding RNA sequences consisting of approximately 22 nucleotides, playing crucial roles in biological and pathological functions. Among these, miR-33a/b is particularly associated with metabolic diseases, notably obesity and atherosclerosis. In this pilot case-control study, 45 subjects were examined, and serum miR-33b levels were measured in three groups: a control group, hypercholesterolemic (HC) subjects without obesity (HC group), and obese subjects without hypercholesterolemia (obese group). Serum miR-33b levels were determined using the real-time PCR method. The expression of miR-33b was significantly higher in the HC and obese groups compared to the control group ($p < 0.001$). The Body mass index (BMI) in the obese group was significantly higher than in the control and HC groups ($p < 0.001$). Additionally, serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) levels were higher in the HC group compared to both the control and obese groups. Our study demonstrated a correlation between serum miR-33b levels and HC and obesity. Finally, the ROC analysis demonstrated that miR-33b had an AUC of 0.74 for identifying hypercholesterolemia and an AUC of 0.76 for identifying obesity, indicating its acceptable diagnostic value alongside traditional markers. Therefore, serum miR-33b levels can be considered as a potential biomarker for obesity and hypercholesterolemia, but these finding are preliminary and further investigation is necessary in larger samples to confirm these associations.

Keywords Atherosclerosis, Obesity, miR-33b, Hypercholesterolemia, HDL-c

*Correspondence:
Beydolah Shahouzehi
bshahouzehi@gmail.com; b.shahozehi@kmu.ac.ir
[1]Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[2]Neuromuscular Rehabilitation Research Centre, Semnan University of Medical Sciences, Semnan, Iran
[3]Cardiovascular Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran
[4]Department of Community Nutrition, School of Nutrition Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
[5]Gastroenterology and Hepatology Research Center, Institute of Basic and Clinical Physiology Sciences, of Clinical Biochemistry, Kerman University of Medical Sciences, Jahad Boulevard Avicenna Avenue, Kerman 7619813159, Iran

Introduction

MicroRNAs (miRNAs) are non-coding, conserved RNA sequences consisting of a single strand of approximately 22 nucleotides. They play an important role in cellular processes by modulating physiological homeostasis and are implicated in various pathological conditions [1–4]. By binding to specific regions of their targets, miRNAs can either suppress the translation of the target gene or degrade its mRNA [5, 6]. Cholesterol is a vital molecule for cells, essential for maintaining the integrity of the cell membrane and the function of membrane proteins. Considering that hypercholesterolemia is a major contributor to cardiovascular diseases (CVD) [7], controlling cholesterol levels is important to safeguard health and prevent CVD [3]. Obesity is a complex disease characterized by the excessive accumulation of lipids in the body [2]. The prevalence of obesity has reached epidemic levels, posing a significant challenge as it is a risk factor for various other diseases, including metabolic disorders, diabetes, and CVD. The management and treatment of obesity and related conditions present a serious challenge due to the increasing prevalence over time [5, 8].

The effects of miRNAs can be inhibited, which helps reveal the functions of miRNAs such as miR-33a/b, miR-130b, miR-143/145, and miR-375, which have demonstrated significant effects on atherosclerosis and obesity [5, 9–11]. One miRNA closely associated with CVD and obesity is miR-33a/b, which plays a regulatory role in cholesterol and lipid metabolism [3, 12]. Studies have shown that miR-33a and miR-33b are intronic miRNAs encoded within the intronic regions of the sterol response-element binding protein-2 (SREBP-2) and SREBP-1 genes, respectively [13, 14]. SREBP transcription factors play a key role in regulating cholesterol levels and fatty acid metabolism; their dysregulation is linked to conditions such as obesity, atherosclerosis, and metabolic syndrome [5].

The involvement of miRNAs in various human diseases, particularly cardio-metabolic conditions such as metabolic syndrome, obesity, and coronary heart disease has been established [3, 7, 15]. miR-33a/b downregulates ATP-binding cassette subfamily A member 1 (ABCA1) and influences Carnitine O-Octanoyltransferase (CROT), Hydroxy acyl-CoA dehydrogenase subunit beta (HADHB), and Niemann-Pick C1 (NPC1) genes, which encode important enzymes involved in fatty acid metabolism and cholesterol transport. Inhibiting miR-33a/b in non-human primates led to elevated levels of plasma high-density lipoprotein cholesterol (HDL-c) [3, 12–19]. Studies have indicated that miR-33b promotes the transport of cholesterol from macrophages to plasma and liver, suggesting that inhibiting miR-33 could potentially prevent foam cell formation and atherosclerosis [19]. Price et al. (2021) observed that hepatic miR-33 knockout enhanced metabolic balance and liver function without affecting body weight or atherosclerosis improvement. Notably, hepatic miR-33 deficiency increased reverse cholesterol transport capacity and HDL-c levels in mice [20]. Conversely, Karunakaran et al. demonstrated in a mouse model of diet-induced obesity that therapeutic silencing of miR-33 boosted whole-body oxidative metabolism but did not affect metabolic dysregulation. Their findings suggest that reducing miR-33 may attenuate atherosclerosis as a safe treatment option [19].

Recent studies suggest that inhibiting miR-33 may have detrimental effects on lipid and insulin metabolism in mice [3, 12], leading to obesity and insulin resistance in miR-33-deficient mice fed a high-fat diet (HFD) [1]. It has been reported that levels of miR-33a/b were elevated in the serum of children with hypercholesterolemia compared to healthy control subjects [7]. Additionally, circulating miR-33 was found to be higher in obese individuals compared to the control group, although this increase was not statistically significant [15]. While inhibiting miR-33 was proposed as a means to raise HDL-c levels and improve atherosclerosis, the results have been conflicting [11, 18–20].

miRNAs play a role in human diseases, and some are being explored as valuable clinical tools. miRNAs can assist in disease diagnosis because they vary in various tissues, such as adipose tissue and liver, linked to obesity, atherosclerosis, and metabolic syndrome [11, 12]. The alterations in miR-33 expression are influenced by different diseases, and determining which disease is most closely associated with changes in miR-33 and potential miR-33 action as a biomarker for metabolic disease remain the questions of interest. In this study, we examined the variations in serum miR-33 levels in a group of obese individuals without hypercholesterolemia and in individuals with hypercholesterolemia who were not obese, comparing them with a control group without metabolic disease. Also, determine the predictive value of miR-33b in these patients.

Materials and methods

Participants

This study is a pilot case-control study which used the information from the individuals who participated in our study were selected from the population-based KERCADRS (Kerman Coronary Artery Disease Risk Factors Study) conducted in Kerman province, Iran. The KERCADRS study recruited 5,900 city residents aged 15 to 75. Three groups were defined: a control group, HC group (participants with hypercholesterolemia without obesity), and an obese group (obese individuals without hypercholesterolemia). All participants in the KERCADRS study were informed about the study protocols, and written informed consent was obtained from each participant

[20]. The current study was approved by the Ethics Committee of Kerman University of Medical Sciences (Ethics approval code: IR.KMU.REC.1396.2486), and all procedures were conducted in accordance with the standards outlined in the Declaration of Helsinki (1964). The participants' data were obtained from November 11, 2019, to March 10, 2020. The authors had no access to information that could identify individual participants during or after data collection.

Demographic, clinical, and anthropometric measurements

The following parameters were extracted from the KERCADRS main database at the Kerman Physiology Research Center for the participants in this study: sex, age, body mass index (BMI) [defined as weight (kg) divided by the square of height (m^2)], where according to the World Health Organization (WHO) an overweight person is defined as having a BMI between 25 and 29 kg/m^2, and an obese person is defined as having a BMI ≥ 30 kg/m^2. Hypertension was defined as blood pressure (BP) > 140/90 mmHg. Total cholesterol (TC) < 200 mg/dL was defined as normal, while levels higher that 220 mg/dl were defined as hypercholesterolemia. Fasting blood sugar (FBS) < 100 mg/dL was defined as normal, and ≥ 126 mg/dL was defined as diabetic. BP was measured in a sitting position after 10 min at rest. The medical history of hyperlipidemia and CVD was recorded by a general practitioner. Smoking status was reported as current smoker if the subject stated that he/she has been smoking cigarette. For participants with a positive drug history, the type of drug was recorded. Hyperlipidemia was defined as serum TC > 240 mg/dL, HDL-c < 35 mg/dL, and triglyceride (TG) > 200 mg/dL. The triglyceride-glucose index (TyG) has been proposed as a surrogate marker of insulin resistance and was calculated as; (Ln) [fasting TG (mg/dL) × FBS (mg/dL) / 2] [4, 10, 21–23].

Diet assessment

Participants in KERCADRS also completed a questionnaire regarding their diet and nutritional habits. The questionnaire and additional information can be found in the main KERCADRS article [21].

Biochemical measurements

A 10 mL blood sample was collected from each participant in a glass tube. The blood sample was left at room temperature for 30 min to allow clot formation. Subsequently, the blood sample was centrifuged at 4000 rpm for 10 min, separating the serum from other cellular components. The collected serum was aliquoted and stored at -80 °C for future measurements. FBS, TG, TC, and HDL-c were measured using a Biochemical Autoanalyzer (Hitachi 902, Roche Diagnostics, USA) [4, 21].

Physical activity

The physical activity of each participant was assessed using the Global Physical Activity Questionnaire (GPAQ) as previously described [21].

Exclusion criteria

Subjects with diseases such as cancer, thyroid disorders, liver diseases, and other malignancies were excluded from this study. Control individuals were selected from participants with a BMI < 25, normal TC, TG, FBS, and BP. Participants with other conditions like cancer, thyroid dysfunctions, and liver conditions were not included in the study. Individuals with hypercholesterolemia and a BMI > 25 kg/m^2, as well as obese subjects with TC > 200 mg/dL, were also excluded.

RNA extraction, cDNA synthesis and quantitative real-time PCR

For RNA extraction, we utilized the Total RNA Purification Micro Kit, extracting miRNAs from serum, including miR-33b, where total and small RNAs < 200 nucleotides were isolated (Total RNA purification micro kit, Cat 35350, NORGEN BIOTEK Corp, Canada). The serum was initially vortexed with RL buffer (175 µl), followed by the addition of 200 µl of 96% ethanol and vertexing for 10 s. The cel-miR-39 RNA from NORGEN BIOTEK (cel-miR-39 spike-in kit; No. 59000) was used as control in this step. The mixture was then applied to a column and centrifuged at 6000 rpm for 1 min at room temperature. The flow-through was discarded, and the column was washed with 400 µl of Wash Solution A, with centrifugation for 1 min at 6000 rpm (room temperature). This step was repeated before a final centrifugation for 2 min at 6000 rpm (room temperature). The column was then placed into an Elution tube provided by the kit, and Elution Solution A (50 µl) was added to the column, followed by centrifugation for 2 min at 2000 rpm (room temperature) and then 1 min at 14,000 rpm. The extracted RNA was used for cDNA synthesis using the Norgen microScript microRNA cDNA Synthesis Kit (NORGEN BIOTEK, 54415, Canada). A 10 µl reaction mix containing the microScript microRNA enzyme mix (1 µl) and the extracted RNA was incubated in a thermocycler (BioRad) at 75 °C for 30 min, 50 °C for 30 min, 70 °C for 15 min, and finally stored at -20 °C until used for real-time PCR. The obtained cDNA was diluted 3-fold with nuclease-free water as per kit instructions and used in the real-time PCR reaction. The real-time PCR for miR-33b measurement involved an initial step at 94 °C for 3 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 30 s using Ampliqon SYBR Green Master Mix with the ABI Step One Plus instrument. Real-time PCR reactions were performed in triplicate, and for the normalization of miR-33b values, cel-miR-39 RNA from NORGEN BIOTEK (cel-miR-39 spike-in kit; No. 59000) was used. According to the kit instructions, NORGEN's

Table 1 Comparison of characteristics of participants across three groups

	Total	Healthy	HC	Obese	*p*-value
	N = 45	*N* = 15	*N* = 15	*N* = 15	
History of hyperlipidemia, n (%)	9 (20.00)	2 (13.33)	5 (33.33)	2 (13.33)	0.11
Taking anti-hyperlipidemic drug, n (%)	4 (44.44)	2 (13.30)	10 (66.70)	2 (13.30)	0.01
History of cardiovascular disease, n (%)	4 (8.89)	0 (0.00)	2 (13.33)	2 (13.33)	0.33
Taking anti-hypertensive drug, n (%)	7 (15.56)	1 (6.67)	3 (20.00)	3 (20.00)	0.51
Taking anti-diabetic drug, n (%)	3 (6.67)	0 (0.00)	2 (13.33)	1 (6.67)	0.34
Current smoker, n (%)	6 (13.33)	2 (13.33)	2 (13.33)	2 (13.33)	1.00
Low physical activity, n (%)	18 (40.00)	4 (26.67)	8 (53.33)	6 (40.00)	0.27

Data are presented as n (%) for categorical measures using Chi-square test

Table 2 Comparison of characteristics of participants across three groups

	Total	Healthy	HC	Obese	*p*-value			
	N = 45	*N* = 15	*N* = 15	*N* = 15	Overall	H vs. HC	H vs. O	HC vs. O
Male	*N* = 21	*N* = 7	*N* = 7	*N* = 7				
Age (years)	49.71 (9.11)	49.20 (10.76)	51.73 (8.40)	48.20 (8.21)	0.56	0.73	0.95	0.55
Weight (kg)	69.98 (16.66)	63.47 (9.15)	57.53 (8.53)	88.93 (10.91)	< 0.001	0.22	< 0.001	< 0.001
Height (cm)	164.89 (9.37)	165.85 (10.01)	161.85 (8.85)	166.99 (9.03)	0.29	0.47	0.94	0.30
SBP (mmHg)	112.67 (14.00)	109.00 (14.17)	115.67 (16.13)	113.33 (11.44)	0.43	0.40	0.68	0.89
DBP (mmHg))	72.89 (9.20)	69.00 (9.30)	72.67 (7.99)	77.00 (9.02)	0.06	0.49	0.04	0.38
FBS (mg/dl)	111.09 (55.97)	89.80 (5.52)	132.33 (76.04)	111.13 (55.34)	0.11	0.09	0.54	0.54
TC (mg/dl)	195.20 (52.68)	156.33 (27.09)	262.00 (15.60)	167.27 (22.59)	< 0.001	< 0.001	0.38	< 0.001
TG (mg/dl)	117.69 (53.66)	72.13 (21.67)	145.40 (42.35)	135.53 (58.29)	< 0.001	< 0.001	< 0.001	0.81
HDL-c (mg/dl)	43.82 (12.30)	40.67 (7.23)	53.60 (13.76)	37.20 (8.58)	< 0.001	0.004	0.63	< 0.001
BMI (kg/m^2)	25.58 (4.94)	22.98 (1.65)	21.92 (2.37)	31.82 (2.25)	< 0.001	0.36	< 0.001	< 0.001
LDL-c (mg/dl)	127.84 (43.37)	101.24 (27.52)	179.32 (21.97)	102.96 (20.27)	< 0.001	< 0.001	0.98	< 0.001
THR	4.61 (1.26)	3.98 (1.06)	5.24 (1.54)	4.62 (0.78)	0.019	0.01	0.30	0.33
TyG	8.61 (0.67)	8.04 (0.32)	9.01 (0.60)	8.78 (0.63)	< 0.001	< 0.001	< 0.001	0.46

BMI, body mass index; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; THR, TC/HDL-c ratio; TyG, triglyceride glucose index. Data are presented as Mean (SD) for continuous measures using ANOVA and adjusted for multiple comparisons using Tukey's HSD test

microRNA (cel-miR-39) Spike-In Kit provides quantified synthetic RNA (cel-miR-39) for use in RNA extraction and normalization in RT-qPCR assays. The recovery of cel-miR-39 RNA correlates directly with total RNA recovery. After reverse transcription of the spiked sample RNA, the cel-miR-39 level can be measured using real-time PCR. The expression level of miR-33b was determined using the $2^{-\Delta\Delta Ct}$ method [4].

Statistical analysis

The statistical analysis was conducted using SPSS Version 26 (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM), Stata (Release 17. College Station, TX: StataCorp LLC) and Med-Calc (Version 22, MedCalc Software bv, Ostend, Belgium). All statistical teste were two-tailed, and $P < 0.05$ was considered statistically significant. Data were presented as Mean ± SD, except for the TC/HDL-c ratio (THR), which was expressed as Mean ± SEM. One-way analysis of variance (ANOVA) was utilized to assess the differences in mean miR-33b and other demographic and clinical variables among the three groups, followed by Tukey's test for pairwise comparisons. The Chi-square test was employed to analyze frequency differences. In order to determine the correlation between miR-33b levels and other factors the Pearson correlation coefficient was calculated. Three adjustment models were employed: Model 1 Adjusted: for age, sex, physical activity and history of hypercholesteremia, Model 2 Adjusted: for BMI, TyG, TG, Model 3 Adjusted: for TC, HDL-c, LDL-c, THR. The predictive power of BMI, HDL-c, LDL-c, miR-33b, TC, THR, TG, and TyG ratios in identifying obesity and hypercholesterolemia was evaluated using receiver operating characteristic (ROC) curves and the area under the curve (AUC). Sensitivity, specificity, and positive predictive values (PPV) were calculated for each indicator. Optimal cutoff points were determined based on the maximum Youden Index (J), calculated as J = maximum (sensitivity + specificity-1) and PPV was calculated using the formula: PPV = True Positives / (True Positives + False Positives).

Results

Participant characteristics are displayed in Tables 1 and 2, and 3. No significant variances were observed in disease history, medication use (except for taking

Table 3 Frequency of dietary habits across three groups

		Total	Health	HC	Obese	*p*-value
Food	**Consumption**	***N*=45**	***N*=15**	***N*=15**	***N*=15**	
High-fat dairy	Monthly	39 (86.67)	14 (93.33)	12 (80.00)	13 (86.67)	0.56
	Weekly	6 (13.33)	1 (6.67)	3 (20.00)	2 (13.33)	
Low-fat dairy	Monthly	26 (57.78)	13 (86.67)	6 (40.00)	7 (46.67)	0.02
	Weekly	19 (42.22)	2 (13.33)	9 (60.00)	8 (53.33)	
Red meat (Beef & Lamb)	Monthly	38 (84.44)	12 (80.00)	12 (80.00)	14 (93.33)	0.51
	Weekly	7 (15.56)	3 (20.00)	3 (20.00)	1 (6.67)	
White meat (Chicken & poultry)	Monthly	38 (84.44)	14 (93.33)	11 (73.33)	13 (86.67)	0.31
	Weekly	7 (15.56)	1 (6.67)	4 (26.67)	2 (13.33)	
Fish & Shrimp meat	Monthly	18 (40.00)	8 (53.33)	5 (33.33)	5 (33.33)	0.43
	Weekly	27 (60.00)	7 (46.67)	10 (66.67)	10 (66.67)	
Vegetables	Monthly	39 (86.67)	13 (86.67)	12 (80.00)	14 (93.33)	0.56
	Weekly	6 (13.33)	2 (13.33)	3 (20.00)	1 (6.67)	
Fruit	Monthly	42 (93.33)	14 (93.33)	14 (93.33)	14 (93.33)	1.00
	Weekly	3 (6.67)	1 (6.67)	1 (6.67)	1 (6.67)	
Refined grain	Monthly	3 (6.67)	1 (6.67)	1 (6.67)	1 (6.67)	1.00
	Weekly	42 (93.33)	14 (93.33)	14 (93.33)	14 (93.33)	
Legumes	Monthly	44 (97.78)	15 (100.00)	14 (93.33)	15 (100.00)	0.36
	Weekly	1 (2.22)	0 (0.00)	1 (6.67)	0 (0.00)	
Saturated oil	Monthly	14 (31.11)	4 (26.67)	5 (33.33)	5 (33.33)	0.90
	Weekly	31 (68.89)	11 (73.33)	10 (66.67)	10 (66.67)	
Unsaturated oil	Monthly	38 (84.44)	14 (93.33)	12 (80.00)	12 (80.00)	0.51
	Weekly	7 (15.56)	1 (6.67)	3 (20.00)	3 (20.00)	
Butter & cream	Monthly	11 (24.44)	6 (40.00)	1 (6.67)	4 (26.67)	0.10
	Weekly	34 (75.56)	9 (60.00)	14 (93.33)	11 (73.33)	
Sugare	Monthly	40 (88.89)	14 (93.33)	12 (80.00)	14 (93.33)	0.41
	Weekly	5 (11.11)	1 (6.67)	3 (20.00)	1 (6.67)	
Sauce	Monthly	3 (6.67)	0 (0.00)	2 (13.33)	1 (6.67)	0.34
	Weekly	42 (93.33)	15 (100.00)	13 (86.67)	14 (93.33)	
Carbonated drink	Monthly	9 (20.00)	3 (20.00)	1 (6.67)	5 (33.33)	0.19
	Weekly	36 (80.00)	12 (80.00)	14 (93.33)	10 (66.67)	
Dough	Monthly	29 (64.44)	9 (60.00)	10 (66.67)	10 (66.67)	0.91
	Weekly	16 (35.56)	6 (40.00)	5 (33.33)	5 (33.33)	
Fast foods	Monthly	3 (6.67)	1 (6.67)	1 (6.67)	1 (6.67)	1.00
	Weekly	42 (93.33)	14 (93.33)	14 (93.33)	14 (93.33)	
Tea	Monthly	40 (88.89)	13 (86.67)	13 (86.67)	14 (93.33)	0.80
	Weekly	5 (11.11)	2 (13.33)	2 (13.33)	1 (6.67)	
Coffee	Monthly	7 (15.56)	3 (20.00)	3 (20.00)	1 (6.67)	0.51
	Weekly	38 (84.44)	12 (80.00)	12 (80.00)	14 (93.33)	
Nuts	Monthly	22 (48.89)	6 (40.00)	9 (60.00)	7 (46.67)	0.54
	Weekly	23 (51.11)	9 (60.00)	6 (40.00)	8 (53.33)	
Fried foods	Monthly	33 (73.33)	12 (80.00)	9 (60.00)	12 (80.00)	0.36
	Weekly	12 (26.67)	3 (20.00)	6 (40.00)	3 (20.00)	
Boiled foods	Monthly	34 (75.56)	12 (80.00)	12 (80.00)	10 (66.67)	0.62
	Weekly	11 (24.44)	3 (20.00)	3 (20.00)	5 (33.33)	
Alcoholic beverages	Weekly	45 (100.00)	15 (100.00)	15 (100.00)	15 (100.00)	
Foods containing salt	low salt	28 (62.22)	8 (53.33)	11 (73.33)	9 (60.00)	0.52
	normal	17 (37.78)	7 (46.67)	4 (26.67)	6 (40.00)	
Added salt	yes	31 (68.89)	11 (73.33)	9 (60.00)	11 (73.33)	0.66
	no	14 (31.11)	4 (26.67)	6 (40.00)	4 (26.67)	

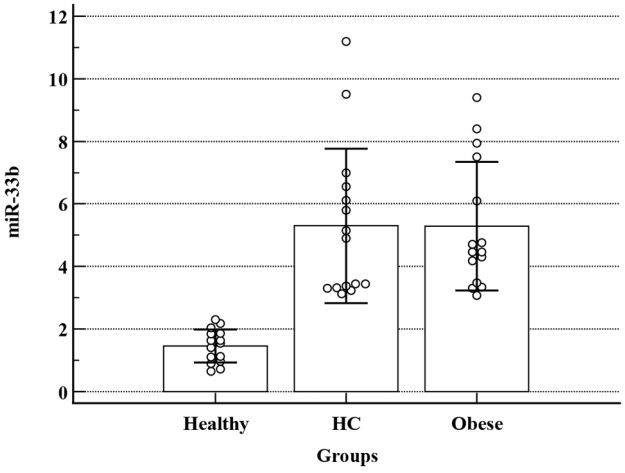

Fig. 1 Serum miR-33 levels quantified in study groups (Healthy control, HC, and obese) by real-time PCR method. Data are expressed as Mean ± SD. $P < 0.05$ was considered significant. The difference between HC and Obese groups with Healthy group was significant ($P < 0.001$)

Table 4 Adjusted partial correlation between miR-33b and other health related factors

	Model 0 r^0	Model 1 r^1	Model 2 r^2	Model 3 r^3
BMI	0.309*	0.354*	-	0.453**
HC	0.353*	0.311	0.570***	0.028
HDL-c	0.121	0.050	0.169	-
LDL-c	0.299	0.327*	0.479**	-
Obese	0.352*	0.409*	0.058	0.476**
TC	0.373*	0.352*	0.465**	-
THR	0.254*	0.368*	0.224	-
TG	0.486	0.404*	-	-
TyG	0.400*	0.319*	-	-0.169

Model 0 Crude, no adjustment; Model 1, r1 as the correlation after adjusting for age, sex, physical activity, and history of hypercholesterolemia; Model 2, r2 as the correlation after adjusting for BMI, TyG, and TG; and Model 3, r3 as the correlation after adjusting for TC, HDL-c, LDL-c, and THR

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

anti-hyperlipidemic drug, $p = 0.01$), smoking status, or physical activity across the groups. The HC group had higher usage of anti-hyperlipidemic drugs than the healthy and obese groups (Table 1). In comparison to the healthy group, the obese group exhibited significantly higher weight, BMI, DBP, TG, and TyG levels (Table 2). Similarly, the HC group showed significantly elevated FBS, TC, TG, HDL-c, LDL-c, THR, and TyG levels compared to the healthy group. Furthermore, the HC group displayed higher TC, HDL-c, and LDL-c levels, and lower weight and BMI compared to the obese group. Our findings showed that the two gender did not differ in terms of the studied variables except THR, where the THR changes was not significant in men ($p = 0.46$), but it was significant in women ($p = 0.011$) (Table 2). Serum miR-33b levels in the obese and HC groups were significantly higher than the healthy group ($P < 0.001$) (Fig. 1). No significant differences in food consumption frequency were noted among the groups, except for a higher intake of low-fat dairy in the healthy group (Table 3).

Table 4 presents the Pearson correlation coefficient between miR-33b levels and various factors. After adjusting for age, sex, physical activity, and history of hypercholesterolemia, miR-33b levels showed significant positive correlations with BMI, LDL-c, TC, THR, TG, TyG, HC, and obesity status. Furthermore, miR-33b levels exhibited significant positive correlations with LDL-c, TC, and HC status after adjusting for BMI, TyG, and TG. Additionally, significant positive correlations were found between miR-33b levels and BMI and obesity status after adjusting for TC, HDL-c, LDL-c, and THR.

Figures 2 and 3, along with Table 5, illustrate the AUC, Cut point, Sensitivity, Specificity, and PPV values for the factors. In the identification of hypercholesterolemia, TC and LDL-c showed the best performance, followed by HDL-c and TyG. Conversely, in identifying obesity, BMI had the highest performance, followed by miR-33b and TG. As it is obvious in Table 5; Fig. 3, the specificity and sensitivity for TC and LDL-c are similar, suggesting that the ROC curves for these two factors overlap. It demonstrating that both curves have strong performance. Also, we should consider that the overlapping nature does not compromise the interpretability of the results.

Discussion

Various miRNAs, including miR-33a/b, miR-34, miR-122, and miR-223, play roles in regulating lipid metabolism. MiR-33a/b, in addition to influencing fatty acid oxidation and HDL-c biosynthesis, also regulates fatty acid and cholesterol metabolism [11–14]. MiR-33 is linked to diseases associated with fatty acid, cholesterol, and lipoprotein metabolism, such as obesity and atherosclerosis [2–4]. Given the connection between miR-33, obesity, and hypercholesterolemia, this study aimed to investigate changes in serum miR-33b levels in obese individuals with normal TC levels as well as in individuals with hypercholesterolemia who were not obese (HC). By categorizing participants in this manner, we eliminated the potential interaction effect between obesity and atherosclerosis, allowing for an examination of the individual relationships of these conditions with serum miR-33b levels, isolated from other diseases. However, some studies, including those by Price et al. (2021 and 2024), underscore complex interactions in which miR-33 levels may not consistently correlate with obesity or hyperlipidemic conditions in different populations. In this way Price and colleagues showed that miR-33 knock out in agouti-related protein neurons elevated feeding in mice on a high-fat diet, leading to metabolic dysregulations and obesity. They also reported in 2021 that, despite the beneficial effects of miR-33 knockout in the liver of

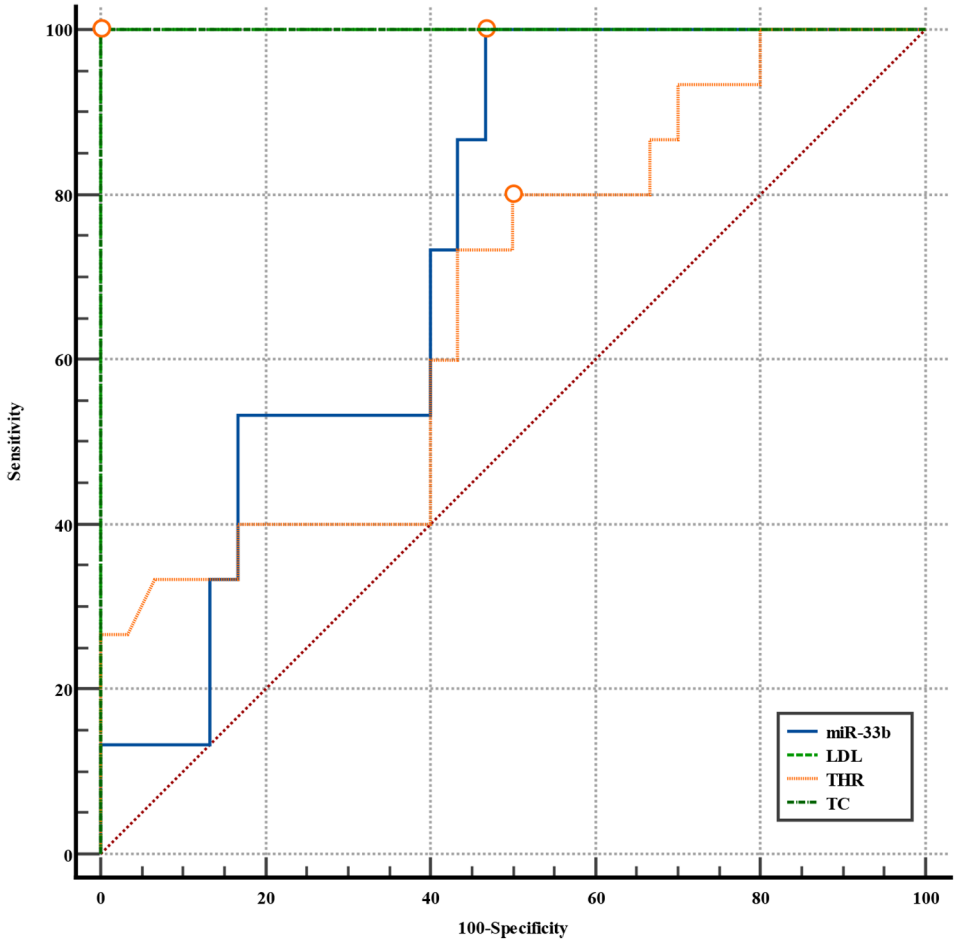

Fig. 2 Comparison of receiver operative characteristic (ROC) curve for miR-33b, LDL-c, THR and TC in hypercholesteremia

mice, the size of atherosclerotic plaque did not change [24, 25].

Our findings revealed that the HC group had higher levels of TC, miR-33b, HDL-c, and LDL-c, and lower weight and BMI compared to the obese group. After adjusting for age, sex, physical activity, and history of hypercholesterolemia, miR-33b levels displayed significant positive correlations with BMI, LDL-c, TC, THR, TG, TyG, HC, and obesity status. MiR-33b levels in the HC and obese groups were higher than in healthy control subjects. While these results align with findings from other studies, most previous research was conducted on laboratory animals [11–14]. Studies by Martino et al. (2015) [7] and AL-Fattli and Al-Tamemi (2021) [15] demonstrated increased serum miR-33 levels in subjects with familial hypercholesterolemia and obese individuals, respectively, consistent with our study. It is important to note that Martino et al. (2015) studied children with FH, who had a lower average age compared to the participants in our study. In the study by AL-Fattli and Al-Tamemi (2021), obese subjects aged 18–66 years were included, which aligns with our study where participants had an average age of 49.71 ± 9.11 years. The increase in miR-33 levels in obese subjects was consistent across these studies [7, 15]. Corona-Meraz et al. (2019) showed that miR-33a and miR33b overexpression was corelated with insulin resistance. They also reported that circulating miR-33b in insulin resistance status did not correlate with lipid profile markers including TG, TC, and very low-density lipoprotein [26]. Unlike their study, we found a correlation between miR-33b and TC. This discrepancy can be explained by the fact that their study included young and aged subjects, whereas our primary goal was to enroll individuals based on their diseases (obesity and hypercholesterolemia). While our results align with earlier studies by Martino et al. (2015) and AL-Fattli and Al-Tamemi (2021), which also observed elevated miR-33 levels in individuals with familial hypercholesterolemia and obesity, contrasting findings from Corona-Meraz et al. (2019) and Price et al. (2021 and 2024) suggest that the relationship may be influenced by demographic factors, metabolic state, or differential expression patterns in various tissues [24–26].

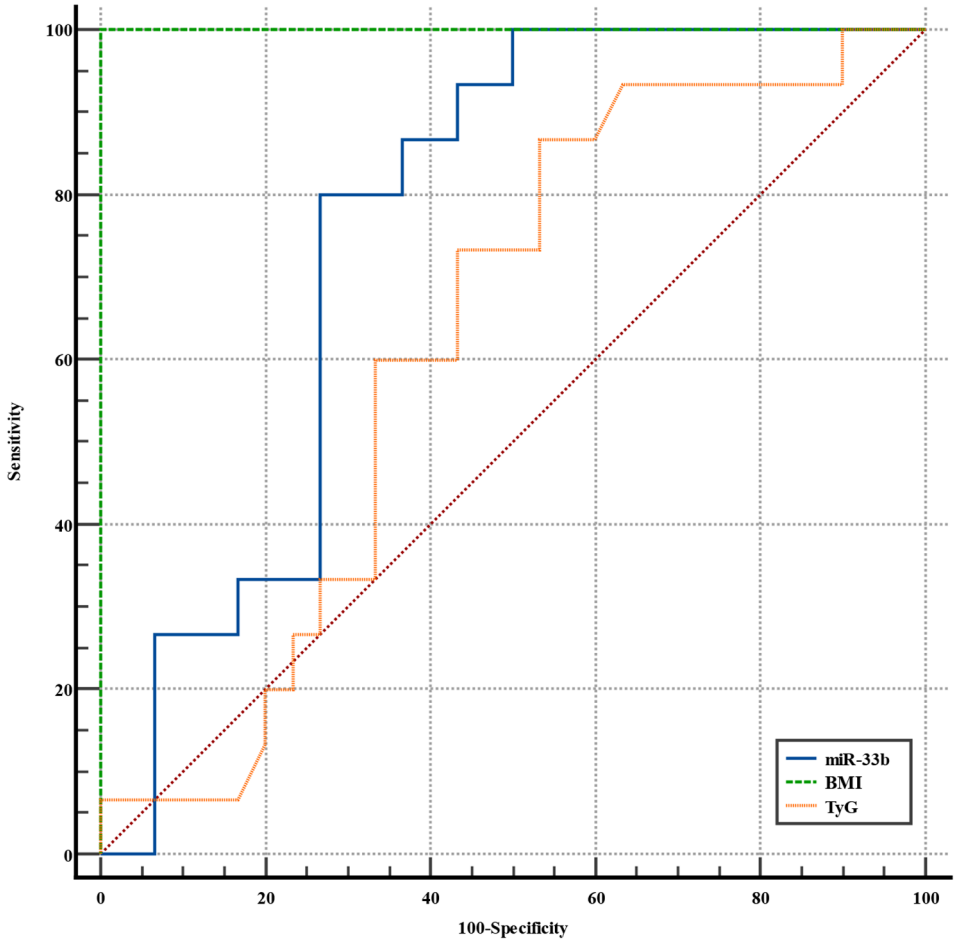

Fig. 3 Comparison of receiver operative characteristic (ROC) curve for miR-33b, BMI and TyG in Obesity

Among the subjects in this study, BMI and serum TC displayed a positive correlation with serum miR-33b levels. Higher BMI is associated with obesity, while increased serum TC is linked to HC status. We observed a direct and strong relationship between these factors. Even after adjusting for BMI, TyG, and TG, the correlation between miR-33b and the HC group, LDL-c, and TC remained significant (r^2: 0.570, 0.479, and 0.465, respectively). This suggests that despite adjusting for obesity-related variables, the relationship between miR-33b and the HC group, as well as cholesterol-related factors, remained significant, indicating a direct and independent relationship. Similarly, after adjusting for TC, LDL-c, HDL-c, and THR, the correlation between miR-33b and obesity and BMI remained significant and stronger (r^2: 0.453 and 0.476, respectively). This indicates that even after adjusting for hypercholesterolemia-related variables, the relationship between miR-33b and obesity and BMI persisted, highlighting a direct and independent relationship. Our study provides evidence linking serum miR-33b levels to both HC and obesity, suggesting its potential role as a biomarker in these conditions. While previous research has focused on miR-33a due to its established relationship with cholesterol regulation [13, 15, 16, 27], our findings indicate that miR-33b may play a significant role in understanding the interplay between obesity and hypercholesterolemia more broadly.

To assess the predictive value of markers for hypercholesterolemia and obesity, we conducted ROC analysis and obtained the AUC values for the factors. In the identification of hypercholesterolemia, TC and LDL-c demonstrated the best performance, followed by HDL-c and TyG. Notably, miR-33b, which exhibited a strong correlation with hypercholesterolemia, had an AUC of 0.74 (95% CI: 0.60, 0.88). Conversely, in the identification of obesity, BMI showed the highest performance, followed by miR-33b and TG. In the context of obesity identification, miR-33b had an AUC of 0.76 (95% CI: 0.62, 0.90), which was higher than TG. Among non-lipid factors such as ABCA1, FBS, DBP, and miR-33, miR-33 stood out with an AUC of 82% as a biomarker for diagnosing atherosclerosis risk [27]. We observed that miR-33b, with an AUC of 0.76, provided an acceptable diagnostic value for obesity compared to BMI. Conversely, regarding

Table 5 Diagnostic performance of different factors for identifying Hypercholesteremia and obesity

	Hypercholesterolemia						Obesity					
	AUC (95% CI)	Cut point	Sensitivity	Specificity	PPV	*P* values	AUC (95% CI)	Cut point	Sensitivity	Specificity	PPV	*P* values
BMI	0.19 (0.06, 0.32)	0.00	0.00	1.00	0.65	0.0001	1.00 (1.00, 1.00)	24.96	1.00	1.00	1.00	0.0001
HDL-c	0.81 (0.64, 0.97)	56	0.60	1.00	0.00	0.0003	0.26 (0.11, 0.41)	73	0.00	1.00		0.0017
LDL-c	1.00 (1.00, 1.00)	137	1.00	1.00	1.00	0.0001	0.25 (0.11, 0.39)	56.20	1.00	0.07	1.00	0.0005
miR-33b	0.74 (0.60, 0.88)	3.07	1.00	0.53	1.00	0.0012	0.76 (0.62, 0.90)	3.45	0.80	0.73	0.87	0.0003
TC	1.00 (1.00, 1.00)	197	1.00	1.00	1.00	0.0001	0.31 (0.16, 0.46)					0.0145
THR	0.67 (0.50, 0.84)					0.051	0.54 (0.37, 0.71)	4	0.87	0.37	0.83	0.639
TG	0.75 (0.60, 0.90)	113	0.80	0.73	0.40	0.001	0.64 (0.46, 0.81)	90	0.73	0.53	0.56	0.122
TyG	0.78 (0.64, 0.93)					0.0001	0.62 (0.45, 0.79)	8.28	0.87	0.47	0.57	0.153

AUC, area under curve; BMI, body mass index; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; PPV, Positive Predictive Value; TC, total cholesterol; TG, triglyceride; TyG, triglyceride-glucose index; THR, TC/HDL-c ratio

hypercholesterolemia, miR-33b had a diagnostic value of 0.74, ranking after TC, LDL-c, HDL-c, and TyG, which had higher AUC values. However, despite having a lower AUC compared to the four aforementioned factors, the AUC of miR-33b remains acceptable, and these factors exhibit strong correlation and a direct metabolic relationship with hypercholesterolemia.

The THR is recognized as a significant and robust risk factor for CVD [28, 29]. In the current study, the THR ratio was found to be 3.98, 5.24, and 4.62 for the control, HC, and obese groups, respectively. Conversely, HDL-c levels in the HC group were measured at 53.6 mg/dl, which was higher compared to the control and obese groups. Despite the elevated HDL-c levels in the HC group, the THR levels were higher in this group than in the other two groups, suggesting an increased risk of CVD in this particular group. The co-occurrence of high miR-33b and THR serum values in the HC and obese groups indicates a potential role of miR-33b in the progression of both atherosclerosis and obesity, even though these conditions were present independently in the subjects under study.

In this study, apart from one instance, the diet of the three groups did not exhibit any significant changes. Notably, individuals in the control group reported higher weekly consumption of low-fat dairy compared to the other two groups. Despite variations in diet, other questionnaire items did not show significant differences. It is commonly recommended for individuals with metabolic diseases to follow a controlled and appropriate diet. Assuming that the control group adhered to a relatively healthy diet, the lack of significant differences may be attributed to individuals in the HC and Obese groups attempting to maintain a healthy diet tailored to their respective conditions, resulting in dietary similarities with the control group.

One significant limitation of this study is the small sample size of 45 participants, which may affect the generalizability of our findings. Small sample sizes often limit the statistical power of a study, making it more difficult to detect significant associations or subtle effects that could be clinically relevant. Additionally, the strict inclusion criteria employed to select individuals with defined conditions of obesity or hypercholesterolemia further narrowed the study population, potentially introducing bias.

Moreover, our primary focus on examining miR-33b levels may have led us to overlook the potential roles of other microRNAs that are also involved in the intricate biological pathways associated with obesity and hypercholesterolemia. This narrow scope raises concerns regarding the comprehensiveness of our findings and limits our ability to draw definitive conclusions about the broader microRNA landscape in these contexts.

It is worth noting that some studies have utilized sample sizes comparable to ours [30–33], but the limited number of participants in our research suggests that caution should be exercised when interpreting our results. Additionally, the need to investigate the interactions and functions of other microRNAs is imperative, as there are likely multiple factors influencing the biological mechanisms underlying these conditions.

To address these limitations, it is crucial for future research to replicate our study with a larger and more diverse sample size. This would not only enhance the validity of our findings but also facilitate better verification of results across different populations. Furthermore, future studies should consider exploring a wider array of miRNAs implicated in obesity and hypercholesterolemia. Adopting such an approach would provide a more comprehensive understanding of the molecular mechanisms involved, potentially leading to the identification of new biomarkers and therapeutic targets for these prevalent health issues. Ultimately, addressing these gaps in knowledge will contribute significantly to the field and may aid in the development of more effective interventions for individuals affected by obesity and hypercholesterolemia.

Conclusion

In conclusion, the close AUC values observed for HC subjects without obesity and obese subjects with normal cholesterol levels (0.74 vs. 0.76) highlight the important contributions of miR-33b in different metabolic contexts. This indicates that miR-33b could provide valuable insights into the pathophysiology of these conditions, beyond traditional risk factors like BMI and LDL-c levels.

Furthermore, the significant correlation of miR-33b with both obesity and hypercholesterolemia suggests a shared molecular pathway that could be important in the progression of CVD [34–36]. Given our observations that LDL-c levels were associated with miR-33b after various adjustments, it emphasizes the need for further investigations into how miR-33b interacts with established cardiovascular risk factors.

Our results encourage further research into the mechanistic roles of miR-33b in obesity and hypercholesterolemia, as well as its potential implications for CVD risk assessment and management. Unraveling these connections could lead to the identification of new therapeutic targets and improve our understanding of the intricate relationship between body weight, lipid metabolism, and cardiovascular health. Thus, miR-33b emerges not only as a valuable biomarker but also as a promising candidate for future studies aiming to address critical health challenges associated with obesity and hyperlipidemia.

Abbreviations

ABCA1	ATP-binding cassette subfamily A member 1
ANOVA	Analysis of Variance
BP	Blood pressure
BMI	Body mass index
CROT	Carnitine O-Octanoyltransferase
CVD	Cardiovascular diseases
FBS	Fasting blood sugar
HDL-c	High-density lipoprotein cholesterol
LDL-c	Low-density lipoprotein cholesterol
HFD	High-fat diet
FH	Familial hypercholesterolemia
HADHB	Hydroxy acyl-CoA Dehydrogenase Subunit Beta
HC	Hypercholesterolemic
KERCADRS	Kerman Coronary Artery Disease Risk Factors Study
miRNAs	MicroRNAs
NPC1	Niemann-Pick C1
SREBP-2	Sterol response-element binding protein-2
THR	TC/HDL-c ratio
TG	Triglyceride
TC	Total cholesterol

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12902-025-01849-9.

Supplementary Material 1

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Author contributions

Y.MA: Conception and design; Experiments; Data Analysis; Writing; Review and Editing. ME: Experiments; Data Analysis; Writing; Review and Editing. HF: Experiments; Data Analysis; Writing; Review and Editing. AJ: Experiments; Data Analysis; Writing; Review and Editing. BS: Conception and design; Experiments; Data Analysis; Writing; Review and Editing.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of Kerman University of Medical Sciences (The Ethic approval Code is IR.KMU.REC.1396.2486) and all procedures was in accordance with standards set by the Declaration of Helsinki (1964). Written informed consent form was obtained from all subjects.

Consent for publication

Not applicable.

Clinical trial number

Not applicable.

Competing interests

The authors declare no competing interests.

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