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Original article

Circulating microRNAs and adipokines as markers of metabolic syndrome in adolescents with obesity

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SUMMARY

Background: Circulating microRNAs (miRNAs) as valuable biomarkers yielded important insights into the pathogenesis of obesity.
Aim: This study aimed to describe the circulating miRNA profile for adolescences and its association with the circulating levels leptin and adiponectin according to specific degree of obesity.
Methods: RT–PCR and immunoassay analysis were used to study circulating miRNA profile, adipokines; adiponectin (A), leptin (L), and L/A ratio as well as other factors of metabolic syndrome (MS) in 250 adolescents with severe obesity.
Results: In morbidly obese adolescents, we identified at least 10 circulating miRNAs, including increased concentrations of miRNAS; miR–142-3p, miR-140-5p, miR-222 miR-143, miR-130, and decreased concentrations of miR-532-5p, miR-423-5p, miR-520c-3p, miR-146a, and miR-15a, which were strongly linked to measures of BMI, WHtR, adipokines; adiponectin, leptin, L/A ratio, and other MS related biomarkers such as FBS, insulin, HOMA-IR, C-peptide, and circulated plasma lipids such as TG, HDL-C, and LDL-C.
Conclusion: Circulating miRNAs showed significant association with plasma levels of adipokines; adiponectin, leptin, and L/A ratios in adolescents with severe obesity. The study provides that regulation of miRNAs expression is associated with adipokines, and other related MS metabolic factors. Thus, early detection of any changes in circulating miRNAs profiles may play a promising role in identifying obese children or adolescents who may suffer from severe metabolic syndrome.

1. Introduction

Obesity considered one the most severe public health problems which affect both the entire human life. It is rapidly arriving to its higher epidemic proportions among children and adolescences globally [1–3]. Most studied, showed that economic, social, and lifestyle changes were significantly associated with obesity, whereas abundant of calorie-rich food and low physical activity were the most characteristics associated with obesity [4,5]. In children and adolescents, higher frequency of obesity was observed worldwide which strongly associated with obesity related consequences in adulthood [2,3,6,7].

Children and adolescences with obesity are easily susceptible to severe health complications such as metabolic syndrome, cardiovascular diseases, and increased adult morbidity and mortality [8–10]. Elevated body mass index (BMI) was shown to be associated with chronic diseases particularly, diabetes, dyslipidemia, hypertension, heart disease, fatty livers, and some types of cancer [11–13]. Metabolic syndrome (Mets) is the most predisposing risk disease referred by a cluster of cardiometabolic risk factors including abdominal obesity, dyslipidemia, hyperglycemia, and hypertension [14]. It significantly increases cardiovascular disease (CVD) and other causes of mortality by 1.5–2 folds in both adults and the pediatric age groups [15–17].

The role of adipose tissue as an active potential contributor in controlling the physiological and pathological processes related to obesity has been extensively studied. As an endocrine organ, adipose tissue was shown to mediate biological effects on normal metabolism and inflammation processes which contributing efficiently in the maintenance of energy homeostasis and, probably,

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pathogenesis of obesity-related metabolic and inflammatory complications [18].

A number of obesity-related peptide hormones known as adipokines secreted by adipose tissue have been identified to play a role in metabolic homeostasis as well as in the pathogenesis of Mets [19,20].

Adiponectin and leptin are the most essential adipokines which associate between adiposity and metabolic disorders [21]. Leptin as a multifunctional metabolic regulator plays a role in altering food intake, energy expenditure, and neuroendocrine function as well as an important mediator of pro-inflammatory state associated with metabolic disorders in subjects with obesity [22]. Conversely, the metabolic status can improve by adiponectin via anti-inflammatory, improving insulin-sensitizing and anti-arteriosclerosis effects [23,24]. Obesity in human subjects is commonly associated with elevation in leptin and decrease in adiponectin levels respectively. Thus, an imbalance of leptin-adiponectin regulation have been existed which may be responsible for the development of obesity related complications. As previously reported, either higher leptin or lower adiponectin levels has been showed to be the early indicators of metabolic disorders in both children and adults with obesity [25–28].

The increasing prevalence of the Mets in children and adolescence with obesity which extends significantly predispose for the subsequent development of metabolic diseases in adulthood [17,29]. Thus, to prevent obesity related Mets in children and adolescences, new trends should be addressed to discover biomarkers for the diagnosis and clinical monitoring of obesity related consequences in younger ages.

MiRNAs; a short noncoding regulatory elements (20–24 nucleotides), were shown to play a role in the regulation of gene expression programs [30–32]. Circulating miRNAs are easily estimated in concentrations which differ significantly according to physiological and pathological states. Thus it could be a useful diagnostic biomarkers for characterization of systemic diseases especially obesity and obesity related Mets [33–36].

We hypothesized in this study that circulating miRNAs may have a role in the regulation of adipokines especially the circulating levels leptin and adiponectin in adolescences with obesity. To our knowledge, this technology has not been tested for targeting the potential association of circulating miRNA signature of obesity with adipokines in children and adolescences.

The aim of this study was to describe the circulating miRNA profile for adolescences and its association with the circulating levels leptin and adiponectin according to specific degree of obesity.

2. Materials and methods

2.1. Subjects

This cross sectional study included a total of 250 schoolchildren (boys n = 150 and girls n = 100) aged 12–18 years were randomly invited during September 2016 and May 2017 from different Prep public schools to participate in this descriptive survey analyses. Children with physical inability, genetic disorders, endocrine and cardiovascular disorders, chronic diseases such as diabetes, cardiac, pulmonary and neurological diseases, or acute infections or who received medical therapy that had affected the data were excluded from this study. The sample size of 250 was selected from the list of students in 10 prep public schools in a large geographical area of Mansoura to give estimate power of 96% and a significance level of 0.05 with expected frequency of 3.21%. Based on the ethical guidelines of the 1975 Declaration of Helsinki, the study protocol was reviewed and approved by ethical committee of Rehabilitation Research Chair (RRC), King Saud University, Kingdom of Saudi Arabia, under file number ID: RRC-2015-051. Prior to data collection, written informed consent was obtained from the parents of all participating schoolchildren. Blood sample in heparinized syringe was collected from all subjects and plasma samples were obtained from whole blood following centrifugation for 1 min at 1400 rpm. The samples were kept frozen at 20 °C until use. Demographic and clinical data of the participants are in Table 1.

2.2. Anthropometric measurements

Standardized procedures were used to measure height and weight in all subjects by using a tape measure and calibrated Salter Electronic Scales (Digital Pearson Scale; ADAM Equipment Inc., Columbia, MD, USA) respectively.

Based on specific BMI cut off criteria previously specified [37] for each age and gender, our subjects were classified into three groups; normal weight (n = 50, BMI; 16.2–17.3 kg/m^2), overweight (n = 100, BMI; 17.4–21.45 kg/m^2), and obese (n = 100, BMI; ≥ 22 kg/m2). In addition, Waist-to-height ratio (WHtR) was calculated according previously validated universal WHtR cutoff>0.5 values which significantly used to identify early cardiovascular risk in both children and adolescents whereas all WHtR cut-off values were based on data obtained from schoolchildren internationally [38–41].

2.3. Sample preparation and analysis of circulating microRNAs

2.3.1. Extraction and purification of circulating RNA

For each subject, total RNA was extracted from plasma samples of 300 μL using the TRIzol LS reagent (Invitrogen, Carlsbad, CA). The procedures were performed according to the manufacturer's instructions as previously reported [36]. All plasma samples were plated onto an Agilent RNA 6000 Nano Chip (Agilent Technologies, Santa Clara, CA, USA), then both the integrity and quantity of total RNA were assessed by using an Agilent 2100 Bioanalyzer (Agilent Technologies).

2.3.2. Amplification and detection of microRNAs using real-time qPCR analysis

A ready-made solutions containing the primers and probes for human miR–142-3p, miR-140-5p,miR-222,miR-143, miR-130, miR-532-5p, miR-423-5p, miR-520c-3p, miR-146a, and miR-15a (Applied Biosystems, Foster City, CA) and real-time RT–PCR was estimated using an ABI 7300 system (Applied Bio systems) [42]. In the reverse transcription step, RNA reverse transcription was performed in 40 μl reactions using both the miRCURY LNA Universal RT microRNA PCR, Polyadenylation and cDNA synthesis kit (Exiqon). cDNA was diluted and assayed in PCR reactions according to the protocol previously reported [42].

RNU43 was used as endogenous reference control, and all PCR cycles were performed according to the manufacturer's instructions as previously described [43], whereas the relative quantification of miRNAs was performed by the 2−ΔCt method. To avoid errors and exactly determine cycle threshold mean values for each sample including amplified miRAs and endogenous control, all reactions were run in duplicate.

2.3.3. Assessment of adipokines

All participants were subjected for estimation of adiponectin and leptin levels in plasma samples by specific ELISA kits (R&D Systems®, Minneapolis, USA). To avoid inter-assay variation, all samples were assayed in duplicate according to the manufacturer's instructions. For both adiponectin, and leptin, the detection limit was 5 pg/mL.

Table 1
Baseline of clinical and laboratory characteristics for adolescents with based on obesity scores (n = 250; mean ± SD).

Parameters	Obesity scores			P-value
	Normal (n = 50)[a]	Overweight (n = 100)[b]	Obese (n = 100)[c]	
Age in years	13.8 ± 2.88	13.85 ± 2.89	13.87 ± 2.91	0.245
Female gender	44.0	49.0	29	0.008
BMI	17.4 ± 4.3	21.9 ± 5.7	26.7 ± 8.2	0.001
WHtR	0.42 ± 0.06	0.65 ± 0.04	0.86 ± 0.09	0.001
Diet score	23.4 ± 3.7	29.6 ± 3.6	31.2 ± 3.7	0.001
MVPA (%)	65.3	41.3	32.1	0.001
Blood pressure (%)				0.002
Normotensive	50	79.0	42.8	
Pre-hypertensive	0	15.8	22.0	
Hypertensive	0	5.2	35.2	
FBG (mmol/L)	4.1 ± 0.3	5.3 ± 0.8	5.9 ± 1.6	0.001
Insulin (mU/L)	6.3 (4.1–8.3)	9.4 (6.4 ± 13.71)	14.5 (7.2 ± 19.7)	0.001
HOMA-IR	1.48 (0.84 ± 1.95)	2.93 (1.86 ± 4.81)	4.65 (2.48 ± 6.81)	0.001
C-peptide (ng/mL)	0.63 ± 0.43	1.65 ± 1.2	1.78 ± 1.4	0.01
Triglycerides (mg/mL)	0.58 ± 0.24	1.38 ± 0.63	2.58 ± 0.94	0.001
HDL-cholesterol (mg/mL)	1.7 ± 0.34	1.23 ± 0.18	1.13 ± 0.28	0.005
LDL-cholesterol (mg/mL)	1.8 ± 0.78	2.31 ± 0.74	3.85 ± 0.72	0.001

Abbreviation: BMI, body mass index; WHtR, waist to height ratio; MVPA: Moderate-to-vigorous physical activity; FBG: fasting blood glucose; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance. All values were reported as mean ± SD or median (interquartile range) or percentage. Significance was calculated by ANOVA followed by Student- Newman-Keul's (SNK) post hoc pairwise comparison for age and metabolic parameters, or Chi-square test for residence and physical activity. a, b, and c refers to the difference between the two group after pairwise comparison. Variables were considered significantly different at P < 0.05.

2.4. Assessment of lipid profile and diabetes

Levels of fasting blood sugar (FBG), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assayed by the Hitachi 7060 C automatic. Also, serum insulin and C-peptide were measured using the sandwich biotin-avidin enzyme-linked immunosorbent assay (BA-ELISA). Insulin assay had an inter-assay coefficient of variation (CV) of <9.0% and no cross-reactivity to proinsulin (<0.05%).

2.5. Statistical analyses

The Shapiro–Wilk test was performed for normal distribution of the data and was logarithmically subjected for statistical analyses. To measure the differences between the studied groups of subjects, both Student's t-test and ANOVA followed by Bonferroni's multiple comparison analysis were used. For the different groups, MiroRNAslevels were adjusted for comparison by univariate analysis using a general linear model. Multiple stepwise regressions and Pearson's correlations analysis were used to estimate the associations between MiroRNAs levels, leptin, and adiponectin.

3. Results

Clinical and metabolic characteristics of 250 adolescents with mean range of age 13.8 ± 2.9 years participated in this cross-sectional study is shown in Table 1. The participants were classified according BMI cut off criteria specified for each age and gender into three groups; normal weight (n = 50, BMI; 16.2–17.3 kg/m^2), overweight (n = 100, BMI; 17.4–21.45 kg/m^2), and obese (n = 100, BMI; ≥ 22 kg/m^2). Higher obesity scores; overweight and obesity were reported in 80% of the total subjects (n = 200), most of them are boys (61%, n = 122). Only, 39% of girls showed higher obesity syndromes.

All overweight and obesity subjects had higher serum levels of cardio metabolic factors; there were significant higher changes in the levels of diabetic control parameters (FBS, insulin, HOMA-IR, and C-peptide), as well as lipid profiles (TG, HDL-C, LDL-C) compared with healthy control subjects. Blood hypertensive was also reported in cases with higher BMI as shown in Table 1. In addition, higher serum levels of leptin and L/A ratio, and lower concentrations of adiponectin were significantly reported in overweight (p = 0.01) and obese (p = 0.001) subjects compared to control subjects as shown in Fig. 1.

In overweight and obese subjects, the correlation between serum levels of adipokines and metabolic parameters are shown in Table 2. Leptin, adiponectin and L/A as markers of MS, correlated negatively with BMI, WHtR, FBS, insulin, HOMA-IR, C-peptide, TG, LDL-C and positively with HDL-C.

Regarding to gender effect on adipokines levels, girls with overweight or obesity had no change in serum levels of adiponectin, leptin, and L/A ratio compared to boys of the same group. The data showed that adiponectin, leptin and L/A ration significantly associated with adiposity markers; BMI and WHtR in boys, but not in girls. However, in normal control subjects there were comparable levels of the studied parameters; adiponectin (p = 0.01), leptin (p = 0.001), L/A ratio (p = 0.001) in boys compared to health girls as shown in Fig. 2.

Circulating miRNAs that were associated with obesity and related consequences were estimated by using standard RT-PCR techniques in plasma samples of 250 adolescents with different

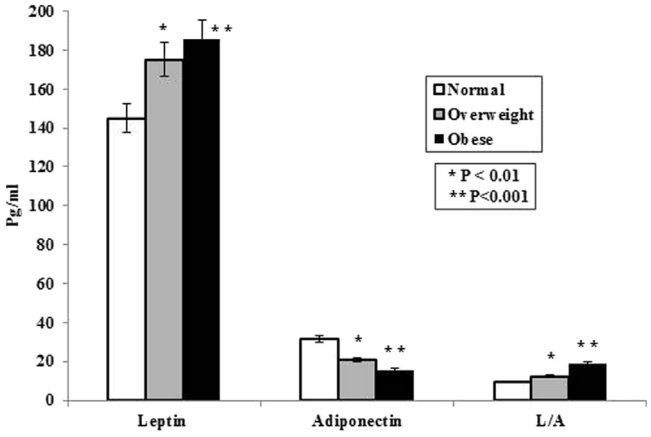

Fig. 1. Plasma levels of adiponectin (A), leptin (L), and L/A ratio (pg/mL) in normal (*n* = 50), overweight (*n* = 100), and obese adolescents (*n* = 100). *p < 0.01 (Kruskal–Walis, Dunn's *post hoc* test).**p < 0.001 (Kruskal–Walis, Dunn's *post hoc* test).

Table 2
Correlation between adipokines biomarkers and clinically studied variables of MS in adolescents with adiposity.

Variables of MS	Adipokines (pg/ml) as markers of MS[a]					
	Leptin (L)		Adiponectin (A)		L/A ratio	
	R	P	R	P	R	P
BMI	−0.38	0.0001	−0.31	0.0001	−0.49	0.001
WHtR	−0.21	0.01	−0.52	0.001	−0.59	0.001
FBS	−0.78	0.001	−0.85	0.001	−0.75	0.001
Insulin	−0.27	0.001	−0.37	0.001	−0.35	0.001
HOMA-IR	−0.31	0.002	−0.47	0.001	−0.28	0.001
C-peptide	−0.78	0.01	−0.58	0.01	−0.039	0.01
TG	−0.26	0.0001	−0.42	0.001	−0.41	0.001
HDL-C	0.64	0.0001	0.48	0.0001	0.67	0.0001
LDL-C	−0.67	0.002	−0.56	0.003	−0.72	0.001

Abbreviation: L/A: leptin to adiponectin ratio, MS: metabolic syndrome. miR, microRNA, BMI: body mass index, WHtR: waist to hip ratio, FBS: fasting blood glucose; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance.
[a] Data are R (spearman).

obesity scores as shown in Fig. 3. Circulating miR−142-3p, miR-140-5p, miR-222 miR-143, and miR-130 were higher in both the obese (n = 100, r = 0.375, p = 0.001) and overweight (n = 100, r = 0.542, p = 0.01) groups compared to healthy control subjects Fig. 2A,B. Whereas, miR-532-5p, miR-423-5p, miR-520c-3p, miR-146a, and miR-15a were significantly lower in the same studied groups than adolescents with the normal weight (Fig. 2C,D).

Both up and down regulated miRNAs levels were positively associated to plasma concentrations of leptin, adiponectin, and L/A ratio as biomarkers of MS in overweight and obese subjects respectively (Table 3). In addition, up regulated miRNAS; miR−142-3p, miR-140-5p, miR-222 miR-143, and miR-130 were positively correlated with BMI, WHtR, diabetic control and lipid profile parameters. Whereas, down regulated miRNAs; miR-532-5p, miR-423-5p, miR-520c-3p, miR-146a, and miR-15a correlated negatively with BMI, WHtR, diabetic control variables, and positively with lipid profile parameters; TG, HDL-C, and LDL-C. All miRNAs showed no significance with age of the studied subjects as shown in Table 4.

4. Discussion

Plasma miRNAs were shown to be essential pivotal biomarkers for the diagnosis, prognosis, and therapeutic value of systemic diseases. In this study, we surveyed a plasma miRNA signature in adolescents with severe obesity. In overweight and obese adolescents, we identified at least 10 circulating miRNAs, including increased concentrations of miRNAS; miR−142-3p, miR-140-5p, miR-222 miR-143, miR-130, and decreased concentrations of miR-532-5p, miR-423-5p, miR-520c-3p, miR-146a, and miR-15a, which were strongly linked to measures of BMI, WHtR, adipokines; adiponectin, leptin, L/A ratio, and other MS related biomarkers such as FBS, insulin, HOMA-IR, TG, HDL-C, C-peptide, and LDL-C.

The expression of miR−142-3p, miR-140-5p, miR-222 miR-143, miR-130 were shown to correlate with the correct development of hematopoietic lineage−specific cells with full consideration as a markers of many diseases particularly those of chronic inflammation, vascular damage, obesity [36,43−46].

Consistent to previous studies, our findings revealed a close relationship between increased plasma concentrations of miR−142-3p, miR-140-5p, miR-222 miR-143, miR-130 with obesity measures among adolescents, further demonstrated by decreased

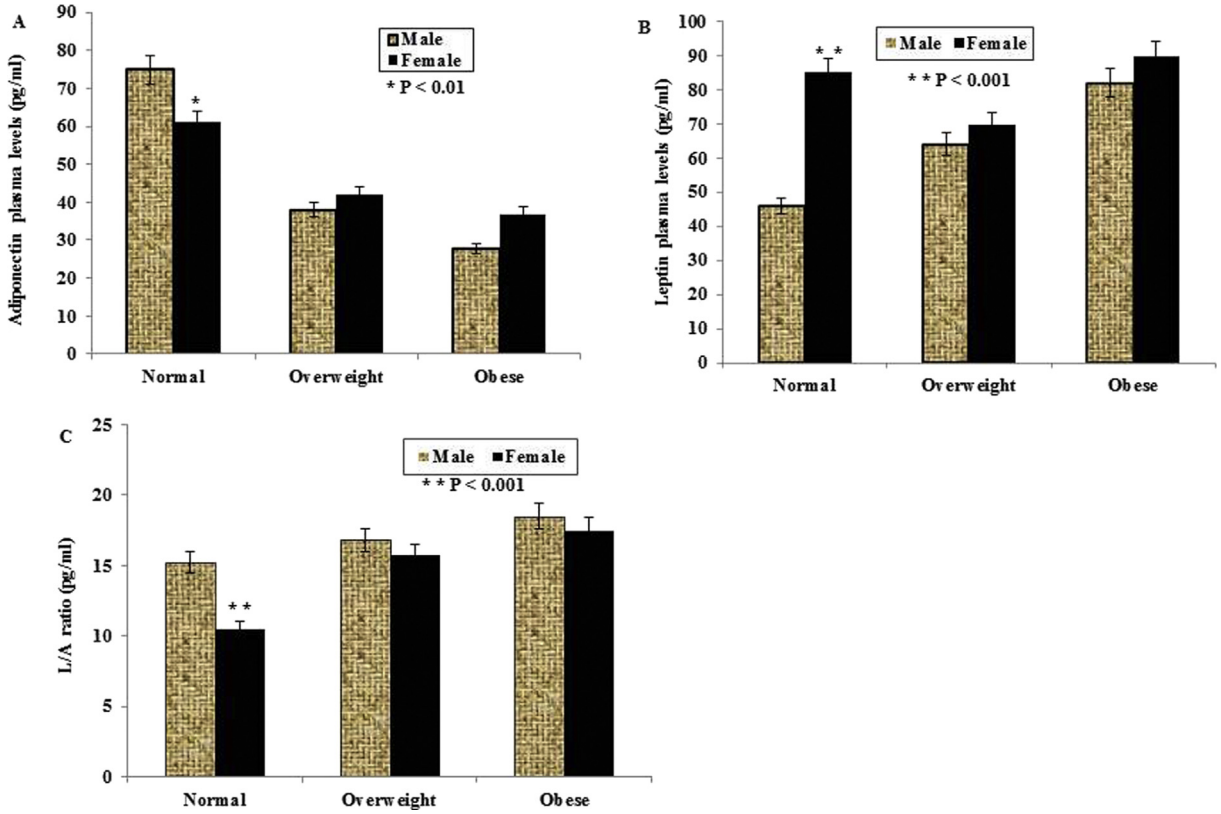

Fig. 2. Effect of Gender on plasma levels of adipokines (pg/mL) in normal (n = 50), overweight (n = 100), and obese adolescents (n = 100). A, adiponectin (A); B, leptin (L); C, L/A ratio. *p < 0.01.**p < 0.001 Mann−Whitney test.

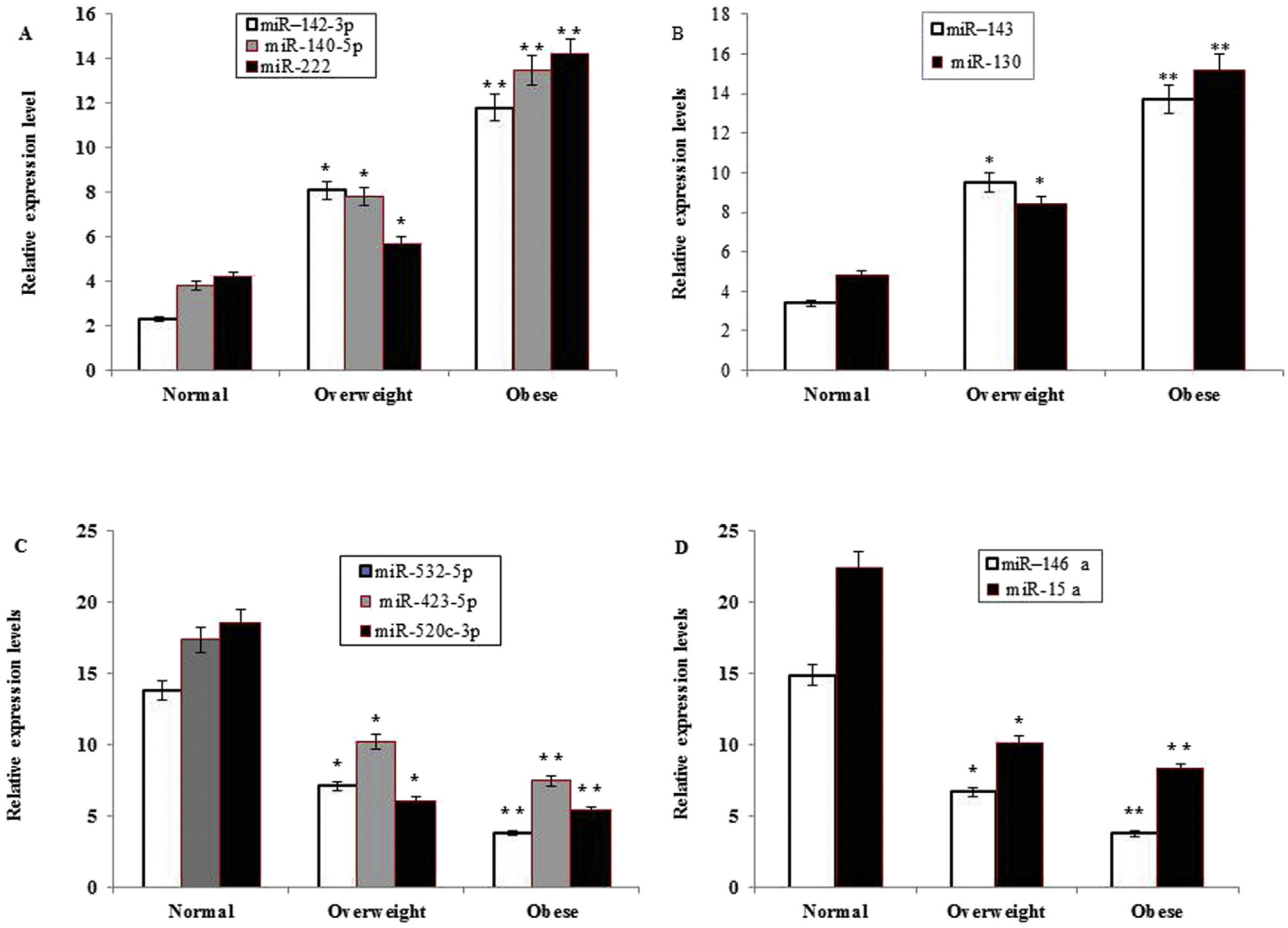

Fig. 3. Screening candidate circulating miRNAs in adolescents with varying obese scores. Data are represented as the mean ± SD. Up regulation of the expression of miRNAs; miR−142-3p, miR-140-5p, miR-222 [A] and miR-143, miR-130 [B] in overweight (n = 100, r = 0.542, p = 0.01) and obesity (n = 100, r = 0.375, p = 0.001) subjects compared to control subjects (n = 50). In addition down regulation in the expressions of miRNAs; miR-532-5p, miR-423-5p, miR-520c-3p [C], and miR-146a, and miR-15a [D] were significantly reported in overweight (r = 0.425, p = 0.01) and obese (r = 0.362, p = 0.001) adolescents compared to normal subjects. *P < 0.01 and * *P < 0.001 vs. control, unpaired t-test. Only data with a significant difference are shown in the figure.

circulating miR-142-3p upon weight loss in previous research studies [43–46]. Previous, studies also reported a marked decrease in the levels of miR-140-5p in plasma samples of obese subjects after induced weight loss. This greatly suggests the association of adiposity markers, fat body and related adipokines with the expression of these circulating miRNAs especially miR-142-3p and miR-140-5p in subjects with severe obesity [45,46].

Similarly, in across-sectional validation study [47], 15 specific circulating miRNAs disclosed and were significantly deregulated in childhood obesity of both boys and girls, including the decreased miR-221 and miR-28-3p and increased concentrations in plasma of miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, and miR-423-5p (all $P = 0$ 0.0001).

Matched with our results, the circulating concentration of these miRNAs correlated significantly with BMI, WHtR, and other measures of obesity such as fat mass %, and with laboratory parameters such as FBS, insulin, HOMA-IR, C-peptide, and circulating lipids TG, HDL-C, and LDL-C in concordance with anthropometric associations [46,47].

Also, the levels of these circulating miRNAs changed significantly and differentially regarding to an increase or decrease in normalized weight among younger ages with obesity [47]. Thus, early estimation of abnormal profiles of circulating miRNAs may have a promising strategy in specifying obese children severe metabolic syndrome.

Compared to our results, several other studies reported significant decrease in the levels of miR-532-5p, miR-423-5p, miR-520c-3p, miR-146a, and miR-15a in plasma samples of subjects with severe obesity [48–50].

Table 3
Correlation between circulating miRNAs concentrations and adipokines biomarkers in adolescents with adiposity (n = 250).

Whole cohort (qRT-PCR	Adipokines (pg/ml) as markers of MS[a]					
	Leptin (L)		Adiponectin (A)		L/A ratio	
	R	P	R	P	R	P
Up regulated miRNAs (↑)						
miR-142	0.35	0.001	0.32	0.001	0.64	0.002
miR-140-5p	0.25	0.003	0.44	0.004	0.51	0.005
miR-222	0.21	0.01	0.43	0.01	0.28	0.02
miR-143	0.51	0.001	0.58	0.001	0.48	0.001
miR-130	0.39	0.002	0.37	0.001	0.35	0.004
Down regulated miRNAs (↓)						
miR-532-5p	0.41	0.002	0.45	0.001	0.49	0.001
miR-423-5p	0.31	0.01	0.37	0.003	0.46	0.001
miR-520c-3p	0.53	0.002	0.59	0.002	0.62	0.002
miR-146a	0.21	0.001	0.26	0.001	0.29	0.001
miR-15a	0.48	0.004	0.56	0.002	0.51	0.001

Abbreviation: L/A: leptin to adiponectin ratio, MS: metabolic syndrome. miR, microRNA.
[a] Data are R (spearman).

Table 4
Correlation between circulating miRNAs concentrations and clinically studied variables of MS[a] in adolescents with adiposity (n = 250).

Whole cohort (qRT PCR)[a]	Age	BMI	WHtR	FBG	Insulin	HOMA-IR	C-peptide	TG	HDL-C	LDL-C
Up regulated MiRNAs										
miR-142	−0.21[d]	0.54[c]	0.59[b]	0.29[c]	0.22[c]	0.32[c]	0.28[b]	0.21[c]	0.42[c]	0.32[c]
miR-140-5p	−0.26[d]	0.42[c]	0.65[b]	0.34[c]	0.27[c]	0.34[c]	0.38[b]	0.19[c]	0.39[c]	0.35[c]
miR-222	−0.31[d]	0.43[c]	0.75[b]	0.18[c]	0.11[c]	0.13[c]	0.41[b]	0.23[c]	0.46[b]	0.36[b]
miR-143	−0.12[d]	0.38[c]	0.51[b]	0.21[c]	0.28[c]	0.33[c]	0.25[b]	0.26[c]	0.38[b]	0.37[b]
miR-130	−0.18[d]	0.31[c]	0.47[b]	0.26[c]	0.23[c]	0.35[c]	0.52[b]	0.25[c]	0.49[c]	0.29[c]
Down regulated mIRNAS										
miR-532-5p	−0.06[d]	−0.48[c]	−0.18[c]	−0.14[b]	−0.35[b]	−0.25[c]	−0.18[b]	0.24[b]	0.38[b]	0.21[b]
miR-423-5p	0.12[d]	−0.65[c]	−0.23[c]	−0.29[b]	−0.37[b]	−0.31[c]	−0.31[b]	0.27[b]	0.59[b]	0.23[b]
miR-520c-3p	0.16[d]	−0.38[c]	−0.34[c]	−0.32[b]	−0.41[b]	−0.35[c]	−0.37[b]	0.31[b]	0.65[b]	0.22[b]
miR-146a	0.11[d]	−0.46[c]	−0.36[c]	−0.38[b]	−0.48[b]	−0.43[b]	−0.46[b]	0.54[b]	0.71[b]	0.216[b]
miR-15a	0.013[d]	−0.41[c]	−0.42[c]	−0.28[b]	−0.56[b]	−0.46[b]	−0.29[b]	0.61[b]	0.38[b]	0.193[b]

Abbreviation: L/A: leptin to adiponectin ratio, MS: metabolic syndrome. miR, microRNA.
[a] Data are R (spearman).
[b] (P = 0.001).
[c] (P = 0.0001).
[d] (NS, not-significant).

The miR-520 and miR-15a cluster target genes have overlapping functions in many disease especially human embryonic stem cells, and tumor cells [48,49], they have been highly expressed in the regulation of the cell cycle and apoptosis mechanisms.

Matched with our results, in previous studies, circulating miR-532-5p, miR-423-5p, miR-520c-3p, miR-146a, and miR-15a concentrations were inversely associated with measures of obesity such as BMI, WHtR and other metabolic disorders in subjects with severe obesity [51–54]. Also, previous studies revealed the inverse associations of miR-423-5p expression with obesity measures and markers of metabolic syndrome such as such as FBS, insulin, HOMA-IR, C-peptide and circulating lipids TG, HDL-C, and LDL-C in relation to BMI, WHtR. Also, the data showed that during weight loss of obese subjects the expression of miR-423-5p is restored or up regulated which significantly confirms the associations of these miRNAs in obesity [55,56].

In addition, in this study, changes in plasma levels of leptin, L/A ratio, and adiponectin significantly correlated with serum levels of cardio metabolic factors in overweight and obese subjects. Girls with severe obesity showed no specified change in plasma levels of adipokines compared to boys. However, in normal control subjects there were comparable levels of the studied parameters in boys than girls. The data showed that adiponectin, leptin and L/A ration significantly associated with adiposity markers; BMI and WHtR in boys, but not in girls.

Obesity was shown previously to associate with sever health complications such as insulin resistance, diabetes, and dyslipidemia, and long-term vascular complications [57–59]. In adolescents, obesity was shown to be linked with higher rates of the severe metabolic syndrome [60,61]. Also, the metabolic syndrome increased significantly with increasing insulin resistance, FBS, HOMA-IR, C-peptide, lower levels of adiponectin in subjects with severe obesity as mentioned previously [62].

In other study, leptin-adiponectin imbalance referred as by an increase in L/A level was found to be a better diagnostic biomarker for MS than leptin or adiponectin alone in children and adolescents severe obesity. Whereas, increased levels of L/A ratios were significantly associated with major components of MS biomarkers such as circulating lipid markers TG, HDL-C, and LDL-C, and diabetic control variables in relation to BMI, WHtR [63].

Thus, children and adolescents with severe obesity clinically reported a significant increase in a cluster of cardio metabolic risk factors, including central obesity, glucose intolerance, hypertension and dyslipidemia as well as changes in the levels of adipokines. These changes collectively referred to a significant severe state of metabolic syndrome (MS) which may established to severe insulin resistance (IR), higher C-peptide preservation, and type 2 diabetes (T2D) as well as cardiovascular disease (CVD) [64].

Also, previous studies showed significant association between adiponectin and MS metabolic factors such as CRP, insulin, IR, higher C-peptide and adiposity markers BMI in obese children and adolescents and acknowledged adiponectin as a biomarker of the metabolic syndrome in childhood obesity [65].

Finally, our study signifies significant changes in plasma concentrations of expressed circulating miRNAs in adolescents with severe obesity which correlated positively with adipokines leptin, adiponectin, L/A ratios, and other related biomarkers of MS. In this study, we add to our knowledge the first evidence for association of circulating miRNAs with adipokines as markers of MS associated with obesity in adolescents. Recently, circulating miRNAs pattern was shown to be associated with human obesity and related consequences, and that significant changes in their profiles were significantly observed in before or after weight loss management trials [30,45]. This signifies the potential molecular mechanisms of circulating miRNAs which implicated in accelerating pre-adipocyte proliferation, a myotube glucose intolerance, and controlling of both lipogenesis and adipose tissue immunity/inflammation [66–69]. It is not surprising that miRNAs may also contribute to the regulation of energy balance and metabolic homeostasis, by controlling a wide range of metabolic pathways [70], and that miRNAs can play a role in fat cell regulation via a suppressive effect on fat metabolism by targeting some genes like p38 and MAPK [71,72] which involved in obesity and obesity related metabolic syndrome (MS) and could efficiently predict the future risks of MS in obese children.

The only limitation is our study failed to measure the effect of gender specific up on expression of circulating miRNAs, whereas adiposity relating adipokines showed variations between boys and girls especially in healthy control subjects with normal weights. This may be due to there was no significant changes in adiposity markers in both girls and boys with severe obesity.

5. Conclusion

Circulating miRNAs showed significant association with plasma levels of adipokines; adiponectin, leptin, and L/A ratios in adolescents with severe obesity. The study provides that regulation of miRNAs expression is associated with adipokines, and other related MS metabolic factors. Thus, early detection of any changes in circulating miRNAs profiles may play a promising role in identifying

obese children or adolescents who may suffer from severe metabolic syndrome.

Disclosure

The authors report no conflicts of interest in this work.

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