Correlation of circulating miRNA-33a and miRNA-122 with lipid metabolism among Egyptian patients with metabolic syndrome

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Abstract

Background: Metabolic syndrome is defined as a group of interrelated biochemical, clinical, and metabolic factors that directly increase the risk of cardiovascular disease, obesity, and type 2 diabetes mellitus. MicroRNA-33a (miR-33a) and MicroRNA-122 (miR-122) play a crucial role in various biological processes by regulating the gene expression level through post-transcriptional mechanisms, and alterations of their levels are associated with lipid and glucose metabolic disorders. In the present study, we aimed to investigate the correlation of miR-33a and miR-122 with obesity indices and glycemic parameters in a cohort of Egyptian patients. Quantitative real-time polymerase chain reaction (RT-PCR) using TaqMan assay was carried out to estimate the expression levels of miR-33a and miR-122 in serum samples of 100 patients diagnosed as having metabolic syndrome and 50 healthy controls. All patients (100%) had type 2 diabetes (by both history and laboratory assessment) and 70% were obese (BMI ≥ 30 kg/m²).

Results: Compared to controls, patients had significantly higher serum expression level of miR-33a (p value < 0.001) and miR-122 (p value = 0.0016). miR-33a was less expressed (downregulation expression) with 0.8 fold change in the patient group (obese and diabetic) compared to healthy controls, while miR-122 was highly expressed (upregulation expression) in the patient group of patients with 1.9 fold change. Clinical parameters as body mass index (BMI), wrist circumference (Wc), weight (Wt), and height (Ht) (all p < 0.001); total cholesterol (TC) (p = 0.0115); and triglyceride (TG) (p = 0.0286), all were significantly higher in patients compared to the healthy group. Both miRNAs show statistically significant correlations with clinical and biochemical parameters (p < 0.001).

Conclusions: Circulating miR-33a and miR-122 might be convincing as possible biomarkers for the diagnosis of metabolic syndrome.

Keywords: Metabolic syndrome, MicroRNA, miR-33a, miR-122, Type 2 diabetes, Obesity
microRNAs are small single-stranded non-coding RNA molecules that comprise 20–25 nucleotides with a transcriptional and posttranscriptional regulatory role in gene expression. They are involved in several processes, including lipid metabolism and insulin sensitivity. Studies have found that the expression level of the miRNA reflects its role associated with different disorders. Alterations of miRNA expression levels contributed to various diseases, such as obesity and diabetes mellitus [5]. Importantly, a single miRNA can regulate the expression of hundreds of genes and the expression of a single gene can be regulated by multiple miRNAs. So, miRNAs might act as ideal biological markers for rapid diagnosis, prognosis, and therapeutic mediators in metabolic disorders [6]. Specific miRNAs, including miRNA-33 and miRNA-122, were determined to have important roles in the regulation of lipid and glucose metabolism pathways [7]. miR-33 family is an intronic miRNA encoded by Srebp genes, located in intron-16 within two protein-coding genes for Sterol regulatory element-binding proteins (SREBF), SREBP-2 and SREBP-1 respectively, and it consists of two members named miR-33a and miR-33b [8]. miR-33 plays an important role in the regulation of cholesterol efflux, fatty acid metabolism, and insulin signaling. In concert with their host genes, Srebp2 and Srebp1, miR-33a and miR-33b act to increase intracellular cholesterol and fatty acid levels by balancing transcriptional induction and posttranscriptional repression of lipid metabolism genes [9]. However, miR-33a and miR-33b affect glucose metabolism through pyruvate carboxy kinase (PCK1) and glucose-6-phosphate (G6PC) pathways, and they also control the expression of sirtuin 6 (Sirt6) and insulin receptor substrate 2 (IRS-2) and therefore regulate blood glucose levels [10]. Approximately 75% of total liver miRNA expression belongs to miRNA-122, the most abundant miRNA in the liver. miR-122 plays an essential role in the maintenance of liver function through gene expression regulation, causing reduction of total cholesterol levels, HDL, apolipoprotein, LDL, and apolipoprotein B [11] by affecting regulatory enzymes involved in cholesterol biosynthesis. In glucose pathways, miR-122 reduces lactate production and increases oxygen consumption, by targeting many of glycolytic genes, especially pyruvate kinase (PK) gene [12].

The aim of this study is to investigate the associations of genetic expression of miR-33a and miR-122 in glucose and lipid metabolism as well as their correlations with metabolic syndrome parameters including obesity and type 2 diabetes (T2DM) in a cohort of Egyptian patients.

Methods

Ethics statement

The research protocol was approved by the ethical review committee of the Faculty of Medicine for Girls, Al-Azhar University institutional review board, Cairo, Egypt (AFMG IRB), reference number: 202001093. Sharing was voluntary; an informed written agreement was obtained from each participant before enrollment into the study. Data were anonymous and coded to assure the confidentiality of participants.

Participants

This study enrolled 100 Egyptian patients selected by random sampling technique (males and females), diagnosed as having metabolic syndrome based on the National Cholesterol Education Programmed Adult Treatment Panel III (NCEP ATP III) [14]. They were selected from the outpatient clinic of Endocrinology at Al-Zahraa University Hospital, in Cairo, Egypt. Their age ranges were 34–60 years with a mean average of 48.45 ± 8.06. Fifty apparently healthy volunteers, recruited from paramedical personnel, served as controls of age ranges from 38 to 59 years (47.4 ± 4.2046). Patients and healthy volunteers were subjected to detailed medical and family history. Anthropometric measurements including height, weight, and $BMI$ [weight (kg)/height (m)$^2$] were done using standard protocols [15].

Blood sample collection

Peripheral blood (6 mL) was collected from each participant as follows: 2 mL blood was taken into NaF tubes for blood glucose estimation, 2 mL for HbA1c test, and 2 mL for serum miRNAs. Four milliliters was taken independently into non-gel serum tubes for measuring lipid profile.

Laboratory analysis

For lipid profile, blood was centrifuged at 3000×g for 15 min. Glycosylated Hb (HbA1c) was evaluated by quantitative colorimetry (Stanbio Laboratory, Boerne, TX, USA). Fasting blood glucose (FBG), total cholesterol (TC), high-density lipoprotein (HDL), and triglycerides (TG) were estimated by standard techniques (Olympus automatic analyzer AU 2700, Irish Branch, Ireland) and low-density lipoprotein (LDL) was determined by Friedewald formula ($LDL = TC − TG/5 − HDL$) [16].
miRNA extraction

Serum was extracted by centrifugation at 2000 rpm for 15 min at 8 °C, and the supernatant was transferred into new tubes and stored at −80 °C till further proceeding. miR-33a and miR-122 were isolated from the serum using miRNeasy kit (Qiagen, USA) according to the manufacturer’s instructions. The concentration of the extracted miRNA had been quantified using NanoDrop and stored in aliquots at −20 °C. miR-39 (Qiagen, USA) was used to normalize the expression levels of target miRNAs.

Quantitative reverse transcription

For miRNA-specific reverse transcription, quantitative real-time polymerase chain reaction (qRT-PCR) assays of miR-33a and miR-122 were performed using TaqMan® MicroRNA kit (Lot: 4453320, Applied Biosystems, USA) according to manufacturer’s protocol and reactions were proceeded in step one real-time PCR system (Applied Biosystems, USA). The reaction was performed in a total volume of 25 μL and contained 100 ng of cDNA template, 1X of 20X TaqMan® Gene Expression Assay, 1X of 2X TaqMan® Gene Expression Master Mix, and complete volume up to 25 μL with RNase-free water. Amplification program: 94 °C for 10 min followed by 40 cycles of 94 °C for 20 s then 60 °C for 30 s. Relative quantification (Rq) of miRNAs’ expression was calculated using the 2−ΔΔCT method as 2−(mean patient ΔCt – mean control ΔCt). ΔCt was verified by subtracting the Ct (threshold cycle) values for endogenous control miR-39 from the Ct values for the gene of interest [17].

Statistical analysis

Data were statistically analyzed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 was considered statistically significant. Student T test was used to compare gene expression levels between groups, and correlations between gene expression levels and clinical parameters were analyzed using the Spearman rank correlation coefficient. Clinical data was presented as the mean ± standard deviation (SD) [18].

Results

Clinical and biochemical analysis

This study comprised 100 Egyptian patients, and their age ranged from 34 to 60 years and 50 healthy control of age ranged from 38 to 59 years. HDL shows significantly lower values in patients compared to controls with p value = 0.0394. However, all other parameters showed significantly higher values in patients compared to controls with p value ranges from < 0.001 to 0.0469. Seventy-five percent of patients were females and 25% were males while in control subjects 85% were females and 15% were males. According to body mass index (BMI), 30% of patients were overweight (BMI = ≥25–< 30 kg/m²) and 70% were obese (BMI = ≥30 kg/m²) while in control subjects 44% were of normal weight (BMI = ≥18–< 25 kg/m²) and 56% were overweight. All patients were diabetics with mean glycosylated Hb (HbA1c) 8.475 ± 1.766534 (Table 1 and Fig. 1).

Expression pattern of miRNA-33a in patients

The expression level of miR-33a was significantly higher with a p value < 0.001 in patients compared to the control group. miR-33a showed lower expression (downregulation expression) in patients compared to controls with 0.8 fold change.

Expression pattern of miRNA-122 in patients

The serum expression level of miR-122 was significantly higher with a p value = 0.0368 in patients compared to the control group. miR-122 showed higher expression (upregulation expression) in patients compared to controls with 1.9 fold change.

Expression of miRNA-33a and miRNA-122 in correlation with biochemical criteria

Spearman rank correlation (Rs) of miR-33a and miR-122 with biochemical parameters in patients revealed positive correlations of both miRNAs with glycosylated Hb (HbA1c) (r = 0.885 and 0.965) and fasting blood glucose (FBG) (r = 0.731 and 0.863 of p < 0.001). Meanwhile, there were moderate positive correlations between both miRNAs and lipid profile of patients including triglycerides (TG) (r = 0.342 and 0.291 and p < 0.001), high-density lipoprotein (HDL) (r = 0.149 and 0.268 and p < 0.001), and low-density lipoprotein (LDL) (r = 0.115 and 0.298 and p < 0.001). Correlations of miRNA-33a and miR-122 with BMI were positively highly significant (r = 0.823 and 0.965 and p < 0.001). Sensitivity and specificity of miR-33a were 87% and 83% respectively and for miR-122 were 95% and 92% correspondingly for patients (Table 2 and Fig. 2).

Discussion

Metabolic syndrome (MetS) is a major public health challenge worldwide with an incidence of 20–25% of the world’s population. It is the main cause of obesity, cardiovascular disease, and type 2 diabetes mellitus [19]. The present study comprised 100 Egyptian patients with metabolic syndrome, the majority of patients were females (75% of cases), and the prevalence of obesity was 70%; however, overweight was 30%. These were compatible with numerous studies as determined by Kaur; overweight and obese patients’ cases were 22% and 60% respectively as well as female showed a higher significant difference of obesity more than men [3]. Sliem et al., in 2016, ascertained in their study that women had a higher
prevalence of the MetS than men especially in Iran, India, Oman, Pakistan, Saudi, and Egypt; this might be due to cultural barriers to physical activity that have been reported among women. Another study from Turkey reported the highest prevalence of MetS in women (39.6%) than in men (28%) in the Middle East [2]. In Egypt, Nasr et al., in 2010, claimed that the prevalence of obesity was 70.9% among Egyptian patients included in their study and that women had an elevated prevalence of the MetS than men [20]. Moreover, all analyzed biochemical parameters of our subjects showed statistically significant differences compared to controls. In a study that was done by Mohsin et al., in 2007, on 91 participants (95% females) with age < 20 years presented by diabetes type 2, they declared that their anthropometrical and biochemical features of metabolic

| Characteristics       | Controls (n = 50) | Patients (n = 100) | p-value (< 0.05) is significant | Significance |
|-----------------------|-------------------|--------------------|---------------------------------|--------------|
| miRNA-33a             | 0.0874 ± 0.08755  | 0.0037 ± 0.009579  | < 0.001                         | HS           |
| miRNA-122             | 1.9615 ± 2.62465  | 3.938774 ± 3.938774| 0.0016                          | HS           |
| Age (years)           | 47.4 ± 4.046      | 48.4571 ± 8.06350  | < 0.001                         | HS           |
| Sex (male/female)     | F 75% M 25%       | F 85% M 15%        | –                               | –            |
| BMI (kg/m²)           | Normal weight ≥18–< 25 | 24.965 ± 6.1954   | 34.57 ± 5.01458                 | < 0.001      | HS           |
|                       | Overweight ≥25–< 30  | Normal (n = 22) (44%) | Overweight (n = 30) (30%)  |              |
|                       | Obese ≥30          | Normal (n = 22) (44%) | Overweight (n = 30) (30%)  |              |
| FBG (mg/dL)           | 114.8 ± 47.02168  | 189.878 ± 66.9303  | 0.0140                          | HS           |
| HbA1c (%)             | 5.3 ± 0.433669    | 8.47575 ± 1.766534 | 0.0469                          | HS           |
| TC (mg/dL)            | 172.15 ± 50.2103  | 203.7273 ± 44.2141 | =0.0115                         | HS           |
| TG (mg/dL)            | 133.8 ± 58.01869  | 178.3636 ± 74.463  | =0.0286                         | HS           |
| cHDL (mg/dL)          | 42.5697 ± 9.78827 | 40.4 ± 8.987711    | =0.0394                         | HS           |
| cLDL (mg/dL)          | 100.86 ± 42.86601 | 125.803 ± 38.72177 | =0.0211                         | HS           |

BMI: body mass index, FBG: fasting blood glucose, HbA1c: glycosylated hemoglobin, TC: total cholesterol, TG: triglycerides, cHDL: high-density lipoprotein cholesterol, cLDL: low-density lipoprotein cholesterol, F: female, M: male, HS: high significance. Data presented as mean ± SD value and p value

Fig. 1 Schematic representation of the distribution of controls and diabetic patients with BMI and HbA1. A. Body mass index in healthy controls divided to normal (n = 22, 44%) and overweight (n = 28, 56%) and B. body mass index in patients divided to overweight (n = 30, 30%) and obese (n = 70, 70%) individuals. C. Contribution of glycosylated Hb (HbA1c) in controls (n = 50) and diabetic patients (n = 100)
cholesterol and fatty acids. Thus, endogenous inhibition of AMPK (AMP-activated kinase) plays a critical role in the regulation of lipid metabolism and inhibition of PCK1 (phosphoenolpyruvate carboxykinase 1), which is involved in the regulation of glucose metabolism. A study done by Rashad et al., in 2020, on T2DM Egyptian patients and showed a consistency with our results where miRNA-122 was overexpressed with high significance compared to controls. Conversely, downregulation of its expression leads to a significant increase in glucose production along with activation of the gluconeogenic genes which was negatively regulated by the overexpression of miRNA-33a and Srebpm2 [6].

miR-33a reduces insulin signaling in hepatic cell lines, whereas inhibition of endogenous miR-33a enhances glucose pathway [7]. This was more explained by Ramirez et al., in 2013, as he inspected that overexpression of miRNA-33a in human hepatic cells resulted in inhibition of PCK1 and G6Pc gene expression, leading to a significant reduction of glucose production [10]. Conversely, downregulation of its expression leads to a significant increase in glucose production and results in diabetes mellitus. In 2014, Zhang et al. reported a significant increase in glucose production and though results in diabetes mellitus. In 2014, Zhang et al. reported a significant increase in glucose production and though results in diabetes mellitus. In 2014, Zhang et al. reported a significant increase in glucose production and though results in diabetes mellitus. In 2014, Zhang et al. reported a significant increase in glucose production and though.
could aid for the assessment of the nutritional status and designing a therapeutic strategy suitable for obesity and glucose metabolic diseases. Vasu et al. stated that new approaches integrate RNA sequencing and system biology methodologies will help to elucidate the modulation of gene networks by miRNAs; this may contribute to the regulation of metabolic processes [29].

Conclusions
This study provides clinical evidence that circulating miRNA-33a and miRNA-122 were remarkably correlated to glucose and lipid metabolism. Alteration in the expression of miRNA-33 or miRNA-122 results in obesity and diabetes. Our findings suggested that further demographic studies should be done to understand more
about miRNA-33 and miRNA-122 pathways; this will improve the use of circulating miRNA-33 and miRNA-122 as promising biomarkers of obesity and insulin resistance.

Abbreviations
Ampkα1: AMP-activated kinase; BMI: Body mass index; Ct: Threshold cycle; FBG: Fasting blood glucose; FFA: Free fatty acids; G6PC: Glucose-6-phosphatase; Ht: Height; HDL: High-density lipids; IRS-2: Insulin receptor substrate 2; LDL: Low-density lipids; MetS: Metabolic syndrome; miR-33a: MicroRNA-33a; miR-122: MicroRNA-122; PCK1: Pyruvate carboxy kinase; PK: Pyruvate kinase; RT-PCR: Real-time polymerase chain reaction; Rq: Relative quantification; Sirt6: Sirtuin 6; SD: Standard deviation; SREBF: Sterol regulatory element-binding proteins; TC: Total cholesterol; TG: Triglyceride; T2DM: Type 2 diabetes; Wt: Weight; Wc: Wrist circumference

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Authors’ contributions
MMR performed the molecular study, including miRNA extraction, quantitative reverse transcription, and statistical analysis, and wrote the manuscript. NAMH collected blood, performed anthropometric measurements, and shared in miRNA extraction. IAH collected blood and performed anthropometric measurements and laboratory analysis. ERM performed clinical selection and evaluation of the participants. KSA analyzed and interpreted the results, and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset is generated and/or analyzed during the current study and are not publicly available due to patient privacy but are available from the corresponding author upon request.

Declarations
Ethics approval and consent to participate
The research protocol was approved by the Faculty of Medicine for Girls, Al-Azhar University institutional review board, Cairo, Egypt (ethical review committee), IRB number: 202001093. Sharing was voluntary; an informed written agreement was obtained from each participant before enrolment. Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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