ORIGINAL RESEARCH

Circulating miR-15a and miR-222 as Potential Biomarkers of Type 2 Diabetes

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Background: In recent years, considerable attention has been paid to the role of microRNAs (miRs) as biomarkers in type 2 diabetes (T2D). The aim of the study was to evaluate the expression levels of miR-15a and miR-222 in diabetic, pre-diabetic, and healthy individuals.

Materials and Methods: Ninety individuals, who were referred to the Yazd diabetic center, were enrolled in this study and then classified into three groups as healthy, pre-T2D, and diabetic based on the clinical manifestations. Real-time PCR was performed to explore miRs expression in the plasma samples of the studied population. The correlation between the biochemical characteristic and the expression of these miRs as well as specificity and sensitivity of different clinical markers in healthy and pre-diabetic groups was evaluated.

Results: miR-222 expression was significantly upregulated in the pre-T2D cases compared to the control subjects (P<0.001), while no significant difference was found between the pre-T2D and T2D groups (P<0.05). The expression of miR-15a was statistically downregulated in the pre-T2D and T2D subjects (P<0.05). The receiver operating characteristic (ROC) curve analysis of miR-15a expression with a cutoff point of 1.12 resulted in the area under the curve (AUC) of 85% (95% CI 0.865–0.912; P<0.001) with 84% and 85% sensitivity and specificity, respectively. Similarly, for miR-222, the cutoff point of 4.03 and AUC of 86% (95% CI 0.875–0.943; P<0.001) discriminated against the pre-T2D and control subjects via the sensitivity and specificity of 86% and 87%, respectively. Moreover, miR-15a values showed a negative correlation with FG (R=−0.32, P=0.005); however, miR-222 values were positively correlated with FG (R=0.25, P=0.03) in the pre-T2D group. Furthermore, miR-222 values were correlated with OGTT in the pre-T2D group (R=0.27, P=0.001). In addition, LDL-C had a negative correlation with miR-222 values in the pre-T2D group (R=−0.23, P=0.02).

Conclusion: This study indicated that the plasma expression levels of miR-222 and miR-15a can be considered as non-invasive, fast tools to separate the pre-T2D individuals from their healthy counterparts. Accordingly, this information could be used to predict the development of the disease as well as a direction for optimal therapy, thus refining outcomes in patients with diabetes.

Keywords: type 2 diabetes, miR-15a, miR-222, biomarker, gene expression

Introduction

Type 2 diabetes (T2D) is a common disease that has an increased prevalence rate over the past years as a result of genetic predisposition and lifestyle alterations such as the reduced physical activities and the increased consumption of high-calorie foods.[1] Currently, T2D affects approximately 380 million individuals, which is expected to reach 592 million by 2035.[2,3] Based on the international federation of diabetes atlas, the Middle East and North Africa have the highest prevalence rates of diabetes (10.9%).[4]

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In 2015, about 85% of 30,202 diabetic patients referring to the university-affiliated adult diabetes clinics were diagnosed with T2D. Also, despite the broad availability of medications in Iran, the frequencies of some diabetes complications like chronic vascular problems are relatively high among T2D individuals.[5]

miRs are included in a class of small non-coding small RNAs including 21–25 nucleotides that regulate the expression of various genes by playing a significant role in several biological and pathological processes.[6] Numerous studies have proven the expression of miRs in various tissues and cell types using a tissue-specific expression pattern.[7] Furthermore, the roles of miRs in the regulation of metabolic pathways have been well documented in which they play crucial roles in adipocyte differentiation, energy homeostasis, lipid metabolism, glucose-induced insulin secretion, and inflammation.[8] Therefore, any dysregulation in miRs expression could affect several important cellular functions such as cell cycle regulation, apoptosis, differentiation, and maintenance of the immune system cell, which consequently affect health and disease development.[9] Alteration in miRs is connected to the development, diagnosis, and prognosis of different diseases such as various cancers,[10,11] metabolic,[12] and cardiovascular diseases.[13,14]

Nowadays, the individuals at risk of developing T2D are recognized through testing the readily accessible serum factors such as their levels of glucose, cholesterol, lipoproteins triacylglycerol, and HbA1c. Furthermore, several physical and lifestyle characteristics including BMI, waist-to-hip ratio, blood pressure, sex, food consumption, physical inactivity, and smoking can be also utilized to assess the risk of the developed T2D.[15] Although biomolecules such as cytokines, adipokines, ferritin, and C reactive protein have been addressed as novel biomarkers that are potentially beneficial, their predictive values are similar to the classic ones.[16,17] Besides, neither classic nor novel biomarkers can efficiently predict the T2D emergence.[18] The application of miRs as biomarkers were firstly proposed for recognizing different types of cancers[19,20] and autoimmune diseases.[21]

In case of T2D, different population-based studies have found a close association among different miRs with T2D and its complications.[22–24] The first study suggesting a blood miRs signature was conducted by Zampetaki et al through evaluating plasma samples. Accordingly, in this study, they identified a subset of five miRs (miR-29b, miR-28-3p, miR-223, miR-15a, and miR-126) that demonstrated a dysregulation in 80 pre-diabetic or in participants

with type 2 diabetes.[25] Subsequently, miRs profiling was performed by Kong et al who discovered an elevation in the expression of seven miRs (miR-146a, miR-30d, miR-29a, miR-124a, miR-34a, miR-9, and miR-375) in T2D cases compared to those who were susceptible to T2D.[26] Karolina et al assessed the miRNAs of the whole blood of T2D patients and detected an increase in their miR-192, miR-150, miR-27a, miR-375, and miR-320a levels. Moreover, they also observed a strong association between the elevated fasting levels of glucose and the increment in levels of miR-320a and miR-27a.[27] These pioneering studies additionally justified the prospective of miRs as biomarkers for T2D. Afterward, different experiments have suggested various miRs such as miR-126 and miR-23a, as potential biomarkers of T2D in general population.[17,28]

In this context, the current study was aimed to assess the expression levels of miR-222 and miR-15a in healthy, pre-diabetic, and diabetic individuals to examine the applicability of circulating levels of the aforementioned miRs, as biomarkers, to calculate the risk of the T2D development.

Materials and Methods
Patients

Ninety individuals aged between 35 and 80 years old were enrolled by Yazd Diabetes Research Center. Afterward, they were equally classified into 3 groups as follows: healthy (fasting glucose (FG), ≤5.4 mmol/L, oral glucose tolerance test (OGTT) as 2-h post-load glucose (2hPG), <7.8 mmol/l); pre-T2D (FG, 5.4–6.9mmol/L; 2hPG 7.8–11.0 mmol/l); and diabetes (FG, ≥7.0mmol/L; 2hPG >11.1 mmol/l) groups.[29] An informed written consent form was attained from all the individuals before participating in this research. The study procedure was approved by the ethics committee of the Shahid Sadoughi Medical University (Ethics code: IR.SSU.SPH.REC.1397.028) in terms of the declaration of Helsinki.[30] Control subjects had a body mass index (BMI) <30 kg/m2, had no background of diabetes and were not on medications disturbing glucose metabolism. Moreover, the exclusion criteria were as follows: consumption of anti-diabetic medication; systemic acute or chronic inflammatory diseases; acute respiratory infection; being under physical treatment; history of malignancy and liver cirrhosis; body mass index (BMI) higher than 40 kg/m^2; and diabetic complications including retinopathy, nephropathy, and cardiovascular diseases. Also, standardized methods were applied for

anthropometric values including weight (kg) and height (m). Body mass index (BMI) was then measured as [weight (kg)/(height)^2 (m)^2].

Biochemical Analysis

Venous blood samples were collected from all the studied subjects after 8 h of fasting, which were then handled for biochemical and molecular investigations. For plasma preparation, 2 mL blood was acquired on EDTA-containing tubes followed by centrifugation at 1000 × g for 5 min. Fasting and plasma glucose levels were measured using routine laboratory tests. Subsequently, glycated hemoglobin (HbAlc), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured as previously reported.[31]

Plasma Preparation and RNA Isolation

Circulating miRs were purified from the plasma samples acquired using an EDTA-containing blood tube. Also, venous blood samples of the subjects were taken and then stored on ice until centrifugation. Afterward, to detach the plasma from the buffy coat fractions and the erythrocytes, the samples were instantly centrifuged at 2.000×g for 10 min at 4°C. Subsequently, the supernatant phase (the plasma) was carefully removed and stored at −80°C until further analyses. The plasma samples were then thawed on ice and 500 μL of each one of these plasma samples was removed for RNA extraction. Next, GeneAll kit (General Biosystems, Seoul, Korea) was utilized to extract total RNA including long ncRNA and small ncRNA in terms of the manufacturer's instruction.

CDNA Synthesis and Real-Time qPCR Assay

The contamination content was measured by the A260/A280 ratio using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, USA). Afterward, 10 μL of RNA was added to each reaction. miRs were reversely transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Thermo Scientific,) in terms of the manufacturer's manual. Relative expression was calculated by the $2^{\Delta\Delta Ct}$ method where SNORD47 was utilized as the housekeeping gene. Primer sequences of miR-15a, miR-222, SNORD47 were 5-TAGCAGCACATAATGGTTTGTG-3, 5-CTCAGTA GCCAGTGTAGATCCT-3, and 5-ATCACTGTAAAACCGT TCCA-3, respectively. Also, universal reverse primers were

purchased from the Bonyakhte company (Bonyakhte, Tehran, Iran). In addition, RT-PCR was accomplished using 7 μL of SYBR green PCR Master Mix (Applied Biosystems) and 0.5 μL of primers, 5 μL of cDNA (100 ng/μL), 7 μL of RNAse free water for each one of the reactions with 20 μL of a final volume. RT-PCR reactions included an initial denaturation for 10 min at 94°C, followed by 40 cycles of denaturation for 30 s at 94°C, annealing, and extension for 30 s at 60°C. The melting curve was then considered by increasing the temperature from 75°C to 95°C to assure that no primer dimers or undesirable genomic DNA were amplified. All the reactions were conducted in triplicates on 48-well plates (Applied Biosystems, Step One Plus, USA).

Statistical Analysis

Characteristics of the studied subjects were reported as mean ± standard deviation (SD). Pearson's correlation analysis was also used to compare the differences among logarithmically transformed clinical values (FG, OGTT, TG, HDL, LDL, and HbA1c) and miRs expressions. miR expression values showed a normal distribution as established by the Shapiro–Wilk test. Moreover, one way-ANCOVA test was applied to compare the adjusted means of miRs expressions among the three groups. Statistical analysis was conducted using IBM SPSS Statistics, version 19 (IBM Corp, Armonk, NY, USA) at a statistical significance level of P-value <0.05. ROC curves and the area under the ROC curve (AUC) were constituted to assess the stratification capability of plasma miRs. In terms of the ROC analysis, the best statistical cutoff amounts of plasma miRs were computed, and the sensitivity and specificity were then measured for particular cutoff points. AUCs were also calculated for other clinical features as shown in Table 1.

Results

Table 2 shows the clinical and biochemical parameters of the studied individuals. In this regard, no significant differences were found in the age, sex, BMI, and lipids profile of the studied participants. While HbA1c and FG were significantly higher in the T2D patients as compared to the healthy and pre-T2D individuals (P<0.001). As shown in Table 3, miR-15a values indicated a negative correlation with FG (R=−0.32, P=0.005), while miR-222 values were positively correlated with FG (R=−0.25, P=0.03) in the pre-T2D group. Moreover, miR-222 values were correlated with OGTT in the pre-T2D group (R=0.27, P=0.001). Also, LDL-C had a negative

Table 1 AUCs, Specificity and Sensitivity of Different Clinical Markers Between Healthy and Pre-Diabetic Groups. Comparison Has Been Performed Between Healthy and Pre-Diabetic Group

Clinical Features	AUC (95% CI)	Sensitivity	Specificity	P value
miR-15a and miR-222	73% (0.822–0.903)	73%	70%	<0.001
FG	94% (0.822–0.903)	85%	91%	<0.001
BMI	62% (0.875–0.923)	65%	64%	<0.001
HbA1c	85% (0.765–0.823)	81%	81%	<0.001
TG	67% (0.609–0.734)	67%	63%	<0.001

Abbreviations: FG, fasting glucose; TG, triglyceride; BMI, body mass index.

Table 2 Clinical and Biochemical Characteristics of the Studied Individuals

Variables	Control	Pre-T2D	T2D	P_1 value	P_2 value	P_3 value
Sex (M:F)	21/9	22/8	20/10	NS	NS	NS
Age (years)	51.44±6.04	49.98±9.25	52.42±8.77	NS	NS	NS
BMI, (kg/m^2)	27.60±3.9	28.02±4.14	28.17±5.46	NS	NS	NS
FG, (mmol/L)	4.57±1.32	5.98±1.82	9.87±2.10	<0.001	<0.001	<0.001
OGTT	5.32 ± 0.43	8.42 ± 0.54	14.13 ± 0.45	<0.001	<0.001	<0.001
HbA1c, (%)	5.76±0.41	6.05±0.24	7.34±1.08	<0.05	<0.05	<0.001
TG (mmol/L)	1.58±0.12	2.27±0.40	2.31±0.41	NS	NS	NS
HDL-C (mmol/L)	1.31±0.20	1.30±0.29	1.32±0.34	NS	NS	NS
LDL-C (mmol/L)	2.99±0.24	3.02±0.73	3.17±0.54	NS	NS	NS
miR-15a	2.01±1.74	0.12±2.01	1.04±2.94	<0.001	NS	<0.001
miR-222	2.07±3.01	6.11±9.12	5.04±7.94	<0.001	NS	<0.001

Notes: P1: comparison between healthy controls and pre-T2D; P2: comparison between pre-T2D vs T2D; P3: comparison between T2D patients vs healthy controls.
Abbreviations: NS, not significant; FG, fasting glucose; TG, triglyceride; BMI, body mass index; OGTT, oral glucose tolerance test; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3 Correlation (R Values) Between the Clinical and Biochemical Characteristics with miR-15a and miR-222

Parameters / R Values	Age	BMI	FG	OGTT	HbA1c	TG	HDL-C	LDL-C
miR-15a Healthy	−0.02	−0.21	−0.24	−0.02	−0.10	−0.04	−0.16	−0.02
miR-15a Pre-diabetic	0.09	−0.16	−0.32**	−0.21	0.12	0.13	−0.11	0.13
miR-15a Diabetic	−0.11	0.17	−0.19	−0.13	0.04	0.03	0.01	0.01
miR-222 Healthy	0.01	0.12	0.10	−0.11	−0.02	−0.22	−0.03	0.13
miR-222 Pre-diabetic	0.13	0.05	0.25*	0.27**	0.09	0.24	0.17	−0.23*
miR-222 Diabetic	0.05	0.24	0.16	0.17	0.16	−0.06	0.13	−0.12

Notes: *p values _.05. **p values less than 0.01. Data were evaluated using 2ΔΔCt of miR-15a and miR-222 and variables in log-transformed forms (FG, OGTT, TG, HDL, LDL, and HbA1c).
Abbreviations: FG, fasting glucose; TG, triglyceride; BMI, body mass index; OGTT, oral glucose tolerance test; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

correlation with miR-222 values in the pre-T2D group (R=−0.23, P=0.02).

According to Figure 1, miR-15a expression was remarkably downregulated in the plasma samples obtained from the pre-T2D and T2D (P<0.001) cases compared to the control subjects. However, no significant difference was found between the pre-T2D and T2D group (P<0.05). Likewise, the difference in the expression of miR-222 was also statistically significant among these

three groups. miR-222 expression was found to be higher in the T2D and pre-T2D in comparison with the healthy group (P<0.001), while it was no statistically significant difference between the T2D and pre-T2D groups (P<0.05).

The diagnostic assessment of miR-15a and miR-222 expressions was conducted based on the AUC, specificity, and sensitivity by drawing the ROC curve. To discriminate against the healthy individuals from the pre-T2D subjects, in the case of miR-15a, the optimal cutoff point was 1.12 with
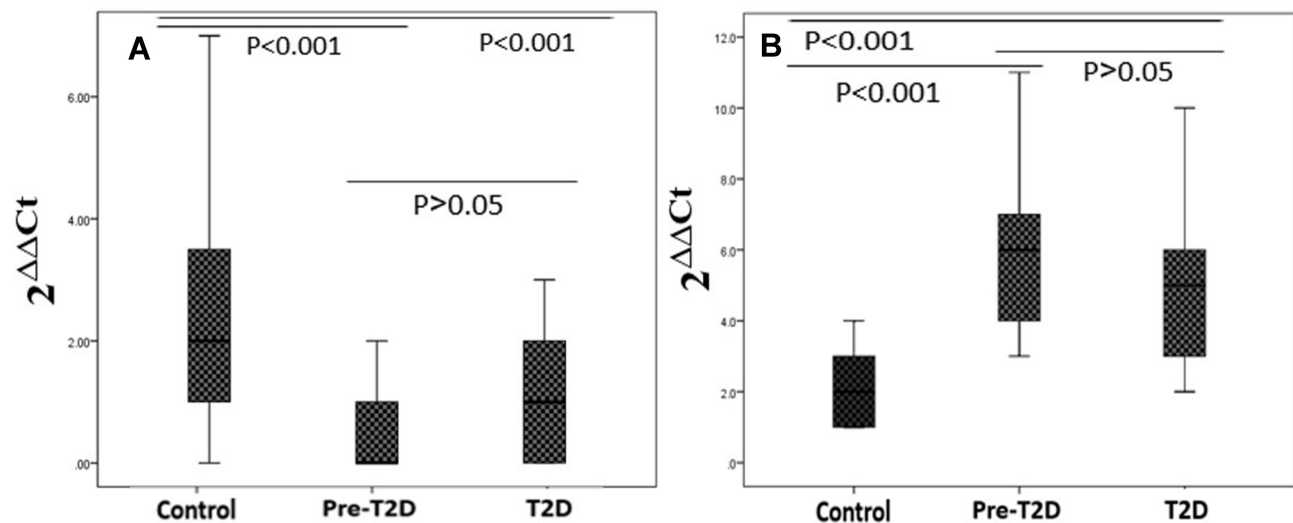
Figure 1 Relative expression of (**A**) miR-15a and (**B**) miR-222. Data are represented as median (interquartile range). One way-ANCOVA test was applied to compare the adjusted means of miRs expression among three groups. Data are depicted by box-and-whisker plots. Boxes indicate the 25th and 75th percentiles, with a solid line within the box that indicates the median.

85% (95% CI 0.865–0.912; P<0.001), 84%, and 85% of AUC, sensitivity, and specificity, respectively. Concerning miR-222, the optimal cutoff point of 4.03 and AUC of 86% (95% CI 0.875–0.943; P<0.001) with the sensitivity and specificity of 86% and 87% were calculated (Figure 2), respectively. As indicated in Table 1, AUC relevant to the combinations of miRs was 73% (95% CI 0.822–0.903) with the sensitivity and specificity of 73% and 70% by comparing the healthy and pre-diabetic groups, respectively. By

comparing between the healthy and pre-diabetic groups, AUC for FG, BMI, TG, and HbA1c were 94%, 62%, 67%, and 85%, respectively.

Discussion

This study is the first experiment that evaluated the expressions of miR-15a and miR-222 in pre-T2D and T2D subjects among the Iranian population. Altogether, these results provide an association between miR-15a and miR-222
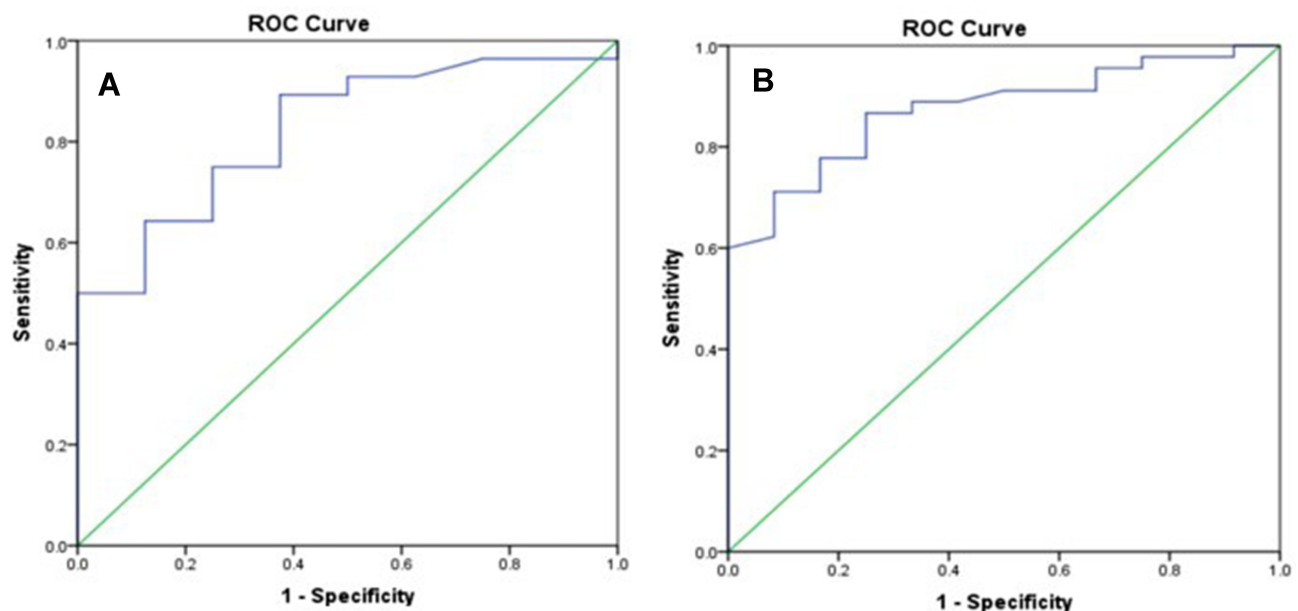

Figure 2 Receiver operating characteristic (ROC) curves of (**A**) miR-15a and (**B**). miR-222 expressions. ROC curves and corresponding AUCs for pre-T2D. The AUC in an established conventional model was 85% (95% CI 0.865–0.912; P<0.001) and 86% (95% CI 0.875–0.943; P<0.001) for miR-15a and miR-222, respectively.

expression levels in diabetic subjects. Levels of miRs expression were also found to be significantly altered in the pre-T2D subjects. Besides, a significant association was also observed between various biochemical parameters and the expression levels of miR-15a and miR-222.

In a study performed by Zampetaki et al, which was conducted in caucasian Italian population, miR-15a has also significantly decreased in plasma before the manifestation of T2D.[25] In this study, 92% of diabetic cases were correctly diagnosed using expression levels of miR-15a as well as the levels of the other miRs (miR-126, miR-320, miR-223, and miR-28-3p). In another study by Flowers et al on the Asian Indians population, a negative relationship was found between plasma miR-15a levels and glycemic progression.[32] Also, an in vitro study revealed a significantly higher miR-15a level after 1-h of glucose exposure, which was declined upon long-term exposure.[33] In a study by Houshmand et al in Denmark, the expression of miR-15a has increased in skeletal muscle of the gestational diabetic and type 1 diabetic subjects compared to healthy individuals. Besides, maternal 2 h post-OGTT glucose levels were significantly associated with miR-15a level.[34] This study also indicated that hyperglycemia can increase offspring miR-15a expression. Moreover, a decreased miR-15a level was also reported in the skeletal muscle of hyperglycemic T2D patients in the Dutch population .[34] Conversely, Wang et al reported the upregulation of miR-15a independent of the other risk factors among the odds of T2D in native Swedish samples.[35]

Concerning miR-222 expression, Shi et al found an increase in miR-222 expression in omental adipose tissues from the patients with gestational diabetes mellitus in Chinese population.[36] Regarding the contribution of obesity to diabetes development, Ortega et al conducted a study on obese Spanish population and found a significant overexpression in miR-222 level.[37] Accordingly, in this study, a discriminant function of miR-222 along with three other miRs (miR-15a, miR-520c-3p, and miR-423-5p) was specific for morbidly obese patients, with a 94% diagnostic accuracy. Furthermore, Li et al showed an upregulation in miR-222 serum levels of T2D Chinese women.[38] On the contrary, investigation of miRs expression pattern in the Italian population performed by De Candia et al revealed a decline in miR-222 expression of T2D subjects, which was inversely correlated with glycated hemoglobin and modulated in the plasma samples of diabetic subjects.[39]

Inconsistent results concerning miR-15a and miR-222 expressions mentioned in previously performed studies can be attributed to different factors. Also, it was obvious that different studies with different population backgrounds (ie, genetic and environmental factors) can affect miRs expression. For example, in a study by Wang et al, it was identified that the expression of several miRs including miR-15a have had different expression patterns in Iraqis and Swedes living in a similar environment.[35,40] On the other hand, the high sequence similarity of miRs family members and the accuracy of various miRs measurement platforms are the other factors accounting for some of the disparities. Additional challenges could also result in more inconsistency such as the definition of disease's stages and specific tissue expression in which the miRs quantification was conducted. As an example, various miRs expression results could be obtained by investigating plasma, serum, and whole blood in a group of T2D subjects with the same ethnicity.[41,42]

Totally, regardless of the high number of studies aimed to identify miRs involvement in diabetes, only a few number of them repeatedly discovered a subset of promising miRNAs. Indeed, most of the reported studies cannot be reproduced using the other sets of specimens. Thus, further studies are required to specify the diagnostic and prognostic potentials of miRs, as a T2D biomarker. So far, several studies have proposed various miRs such as miR-23a, miR-15a, and miR-375 as potential biomarkers for detecting T2D in the general population.[28,43,44] Merely, miR-126 has been repeatedly reported in different studies as a potential predictor of both pre-T2D and T2D individuals.[45,46] Herein, we reported a remarkable difference between expression values of miR-15a and miR-222 in pre-T2D subjects compared to healthy individuals. ROC analysis demonstrated considerable sensitivity and specificity of plasma miR-15a and miR-222 in distinguishing pre-T2D subjects and healthy individuals. AUC pertinent to the combination of miR-15a and miR-222 was 73%, which shows a lower stratification power compared to miR-15a and miR-222. Although AUC of HbA1c is comparable with miR-15a and miR-222; and also, AUCs for BMI and TG have lower values than AUCs of miRs. We also observed an association among miR-15a and miR-222 and FG, OGTT, and LDL-C, which candidates these miRs in diabetes development, while more studies are needed to further clarify these miRs roles.

Concerning miR-222 role in the pathophysiology of diabetes, it has been identified that miR-222 regulates estrogen receptor (ER) and glucose transporter 4 (GLUT4) protein levels in 3T3-L1 adipocytes cells. Moreover, using antisense oligonucleotides of miR-222 to silent miR-222 can lead to an increment in ER expression, GLUT4 translocation from

cytoplasm to the cell membrane, glucose uptake, and an improvement in insulin sensitivity.[36] Furthermore, Tsukita et al showed that bone marrow-secreted miR-222 following the bone marrow transplantation in streptozotocin-induced diabetic rats led to β-cell regeneration as well as amelioration of hyperglycemia.[47] Additionally, overexpression of miR-222 in normal human liver cell line inhibited Coenzyme A oxidase 1, which is the key enzyme in fatty acid β-oxidation, and prevented β-oxidation of fatty acids and remarkably augmenting the triglyceride content.[48]

Alteration in miR-15a expression is associated with various biological processes. In this regard, some studies suggested a critical role for miR-15a in endothelial cell function and angiogenesis in peripheral vascular and cerebrovascular tissues.[49] Upregulation of miR-15a reduces the angiogenesis in pro-angiogenic cells in vitro by targeting vascular endothelial growth factor A (VEGFA), which is associated with myocardial ischemia/reperfusion injury in mice. Moreover, the inhibition of miR-15 in cerebral vascular endothelial cells boosts the pro-angiogenic activity in animal models and in vitro studies.[50] Besides, the downregulation of miR-15a has been found to be correlated with genomic instability and postnatal mitotic arrest of cardiomyocytes, as reported in several studies.[51] Long-term glucose exposure results in the decreased miR-15a expression, which is associated with the decreased insulin expression and biosynthesis. In the same study, miR-15a upregulation led to the promotion of insulin gene expression in mouse insulinoma cells.[33]

Notably, the conventional biochemical indicators for T2D including levels of glucose and HbA1c may predict the initiation of T2D a few years prior to disease emergence. However, these biomarkers are not specific for T2D, which make them impractical to evaluate disease predisposition in the general population. Hence, the discovery of new biomarkers could be helpful in the identification of those people who are at risk leading to an appropriate management of T2D if necessary. The present clinical biomarkers can predict the development of diverse medical disorders such as myocardial infarctions, diabetes, and cancer.[46] The most recent trend in biomarker discovery is searching for sensitive biomarkers applicable for the differentiation of the affected individuals from their healthy counterparts as well as specifying different stages of the disease. Another anticipated merit of these biomarkers is their availability, so that they can be readily attained from body fluids such as saliva, urine, or blood. Moreover, the stability of circulating miRs is remarkable in different situations such as tolerance to ribonucleases and freezing/thawing cycles.[52] For example, plasma or serum specimens can be kept at −80°C for several months with no remarkable degradation.[53]

Conclusions

Overall, this study investigated the expression patterns of miR-15a and miR-222 for possible discernment among healthy, pre-T2D, and healthy subjects. The results revealed different expression patterns for miR-15a and miR-222 in the Iranian population, which can be considered as a valuable cornerstone for upcoming studies on the biomarker capability of these miRs for T2D.

Acknowledgment

Disclosure

The authors declare that they have no conflicts of interests.

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