ORIGINAL ARTICLE

Serum sirtuin 1 protein as a potential biomarker for type 2 diabetes: Increased expression of sirtuin 1 and the correlation with microRNAs

Ozlem Gok[1], Zeynep Karaali[2], Arzu Ergen[3], Sema Sirma Ekmekci[1], Neslihan Abaci[1]

[1]Department of Genetics, Aziz Sancar Institute of Experimental Medicine (Aziz Sancar DETAE), Istanbul University, Istanbul, Turkey, [2]Department of Internal Medicine, Haseki Training and Research Hospital, Istanbul, Turkey, [3]Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine (Aziz Sancar DETAE), Istanbul University, Istanbul, Turkey

Background: Type 2 diabetes (T2DM) is characterized by hyperglycemia and insulin deficiency. Sirtuin 1 (SIRT1), serving as a deacetylase, is critical in the regulation of glucose and lipid metabolism. Recently, a number of studies have been conducted to investigate the role of SIRT1 in the pathogenesis of T2DM. However, there are no sufficient data about the relationship between SIRT1 and T2DM. The aim of this study was to analyze the expressions of microRNAs (miRNAs) (miR-34a, miR-9, miR-132, and miR-181a) involved in SIRT1 regulation and SIRT1 protein in the serum of T2DM patients and controls. **Materials and Methods:** miRNA expressions were determined by real-time polymerase chain reaction, and enzyme-linked immunosorbent assay was used to measure the SIRT1 protein levels in 25 T2DM patients and 25 controls. **Results:** Fasting blood glucose and glycated hemoglobin levels were significantly higher in patients when compared with controls ($P < 0.001$). There was no difference for miRNA expressions between the groups ($P > 0.05$). SIRT1 protein level was significantly increased in patients as compared to controls ($P = 0.044$). Moreover, SIRT1 was negatively correlated with miR-181a ($r = -0.558$, $P = 0.005$) and miR-132 ($r = -0.435$, $P = 0.034$) in patients. **Conclusion:** Obtained results indicate that serum SIRT1 may be a potentially new biomarker for T2DM and also miR-181a and miR-132 may be involved in the development of T2DM by targeting SIRT1. This is the first study reporting on the effects of SIRT1 and related miRNAs in Turkish T2DM patients.

Key words: Diabetes mellitus, microRNA, serum, sirtuin 1

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INTRODUCTION

Diabetes is a progressive metabolic disease caused by the combination of genetic and environmental factors and characterized by hyperglycemia and poses a major threat to human health. According to the World Health Organization, there are over 400 million people worldwide suffering from diabetes and it is estimated that this figure will reach 552 million by 2030.[1,2]

Diabetes can be classified into several categories, but the vast majority of cases of diabetes constitute type 1 diabetes and type 2 diabetes (T2DM).[3] T2DM seen in >90% of patients is characterized by insulin resistance and impaired insulin secretion, leading to macrovascular and microvascular complications.[4,5] Defects in pancreatic β-cells and insulin resistance are the most important features involved in the pathogenesis of T2DM. Depending on the cell-receptor defect, glucose cannot enter into the cell. In particular, the effect of insulin on muscle and adipose tissue is insufficient. The pancreas cannot secrete enough insulin, and hence, glucose production in the liver increases. Furthermore, when insulin-producing β-cells cannot compensate for increased insulin resistance, glucose homeostasis is disturbed, and consequently, T2DM develops.[4,6,7]

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Address for correspondence: Dr. Neslihan Abaci, Department of Genetics, Aziz Sancar Institute of Experimental Medicine (Aziz Sancar DETAE), Istanbul University, Vakif Gureba Caddesi, 34093, Sehremini, Istanbul, Turkey. E-mail: neslihanabaci@gmail.com

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Sirtuin (SIRT) genes have 7 variants (SIRT1–SIRT7) in mammals, and SIRT1 has the most stable deacetylase activity among these genes.[8,9] SIRT1, which acts as a Class I histone deacetylase and helps to maintain the balance between acetylation and deacetylation in posttranslational modifications, plays an important role in the regulation of glucose and lipid metabolism. SIRT1 enhances β-cell protection and insulin secretion in the pancreas, gluconeogenesis and fatty acid oxidation in the liver, lipid mobilization in the adipose tissue, and mitochondrial biogenesis and glucose uptake in the skeletal muscle. In addition, it regulates biogenesis and fatty acid oxidation while reducing the production of reactive oxygen species in mitochondria.[6,10-12]

It has recently been shown that microRNAs (miRNAs) can be secreted by cells and can be detectable in serum and other biological fluids. A number of studies have been conducted on whole blood, serum, plasma, urine, peripheral blood mononuclear cells, and endothelial progenitor cells to identify T2DM-related miRNAs.[13,14] However, knowledge on how T2DM and SIRT1 expression is regulated is insufficient and it has not been fully understood yet.

In this study, we aimed to investigate the expressions of miRNAs (miR-34a, miR-9, miR-132, and miR-181a) involved in SIRT1 protein regulation and SIRT1 protein level in Turkish T2DM patient and control groups.

MATERIALS AND METHODS

Study population

The study groups consisted of 25 T2DM patients (21 females and 4 males and mean age: 51.16 ± 6.82) and 25 controls (12 females and 13 males and mean age: 48.52 ± 9.67). The individuals were examined in (or treated in) the Haseki Training and Research Hospital, Department of Internal Medicine.

After overnight fasting, blood samples of the participants were drawn in plain tubes. The samples were centrifuged for 5 min at 4.500 rpm at +4°C, followed by the removal of serum, and then, serum was stored at −20°C for real-time polymerase chain reaction (real-time PCR) and enzyme-linked immunosorbent assay (ELISA). The following biochemical parameters were determined in both case and control groups by standard laboratory methods in the Haseki Training and Research Hospital: fasting blood glucose (FBG), glycated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol, triglyceride, urea, creatinine, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), folate, Vitamin B12, 25-hydroxyvitamin D, white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), uric acid, sediment 1 h, sodium, potassium, calcium, glomerular filtration rate (eGFR), and C-reactive protein (CRP). Body mass index (BMI) values were calculated by dividing weight by height square (kg/m^2) and categorized well according to the World Health Organization recommendations.

Determination of microRNA expressions

Total RNA was extracted from 200 μL of serum using miRNeasy serum/plasma kit (catalog no: 217184, Qiagen, USA) according to the manufacturer's protocol. The final elution volume was 14 μL. The concentration of all RNA samples was quantified by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and 4 ng of serum RNA containing miRNA was reverse transcribed to cDNA using TaqMan MicroRNA Reverse Transcription Kit (catalog no: 4366596, Thermo Fisher, USA). Then, real-time PCR was performed using TaqMan MicroRNA Assays (catalog no: 4427975, Thermo Fisher, USA) in LightCycler 480 II (Roche, USA). Each sample was run in duplicate for analysis.

As the internal control gene, noncoding small RNA RNU6B (U6) was used, and expression levels of miR-34a, miR-9, miR-132, and miR-181a were calculated using the computed tomography (cycle threshold) method.

Measurement of sirtuin 1 protein levels

Serum samples of individuals were analyzed for the levels of SIRT1 protein using ELISA kit (catalog no: E2557Hu, BT Labs, China) according to the manufacturer's protocol. Briefly, standards and serum samples were added into a 96-well plate. After adding biotin-conjugated anti-SIRT1 antibody and streptavidin-horseradish peroxidase, the plate was incubated for 60 min at 37°C. The wells were then washed five times with wash buffer. Substrate solutions A and B were added, and the plate was incubated for 10 min at 37°C for color development. Finally, the reaction was stopped by the stop solution. The intensity of color in each well was measured in a microplate reader (Multiskan Spectrum, Thermo Electron Corporation) at 450 nm.

Statistical analysis

Statistical analyses were conducted using a standard software package (SPSS 18 for Windows; SPSS Inc., Chicago, IL, USA). Differences in demographic and clinical characteristics were analyzed using Chi-square, Fisher's exact, and Student's *t*-tests. miRNA expression levels and SIRT1 protein levels were compared by Student's *t*-test. Pearson's correlation was used for correlation between SIRT1 and other parameters in groups, and Mann–Whitney

test was used for analysis of risk factors. Student's *t*-test was also used for comparative analyzes in the control group. Fisher's exact test was used if the number in any cell of the 2 × 2 contingency table was <5. $P < 0.05$ was regarded as being statistically significant.

RESULTS

Clinical and biochemical characteristics of type 2 diabetes patients and controls

Clinical and biochemical characteristics of study groups were presented in Table 1. The patients and controls had similar distributions of age. There was no significant difference in age, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, total cholesterol/HDL cholesterol, triglyceride, urea, total protein, AST, ALT, ALP, folate, Vitamin B12, 25-hydroxyvitamin D, RBC, HGB, HCT, PLT, uric acid, sediment 1 h, sodium, potassium, calcium, and eGFR levels ($P > 0.05$). However, FBG ($P < 0.001$), HbA1c ($P < 0.001$), GGT ($P = 0.020$), WBC ($P = 0.002$), and CRP ($P = 0.002$) levels were significantly increased in patients with T2DM compared to controls. Creatinine ($P = 0.004$) and sodium ($P = 0.006$) levels were significantly lower in the patient group, as well. When the study groups were evaluated for smoking ($P = 0.012$), hypertension ($P < 0.001$), and family history ($P = 0.050$), statistical significance was found in the patient group.

MicroRNA expressions and sirtuin 1 protein levels

There was no statistically significant difference when miR-181a, miR-132, miR-9, and miR-34a expression levels were evaluated between the patient and control groups ($P > 0.05$) [Figure 1]. On the other hand, SIRT1 protein level significantly increased in patients as compared to controls ($P = 0.044$) [Figure 2].

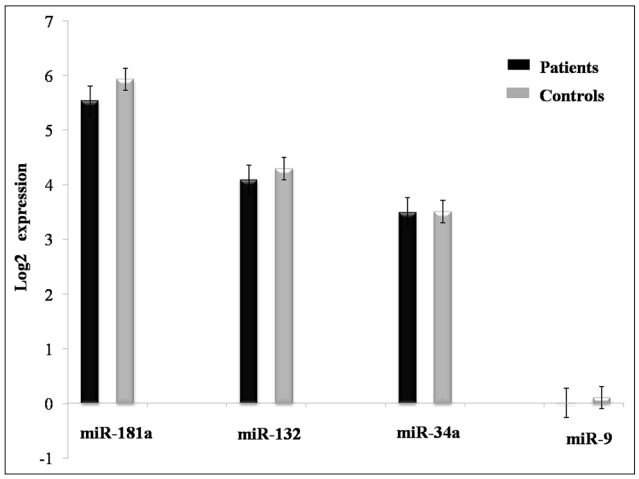

Figure 1: Relative expression of microRNAs in patient and control groups. Statistical evaluation by Student's *t*-test. The results are shown as mean ± standard error of mean in log2 scale. miR-181a ($P = 0.249$), miR-132 ($P = 0.523$), miR-34a ($P = 0.976$), and miR-9 ($P = 0.813$)

Correlation analysis

Pearson's correlation analysis between serum SIRT1 and other factors is summarized in Table 2. Serum SIRT1 was negatively correlated with miR-181a ($r = -0.558$, $P = 0.005$)

Table 1: Demographic and clinical data of patient and control groups

Variable	Patients (*n*=25)	Controls (*n*=25)	*P*
Gender (female/male) (*n*)	21/4	12/13	0.016
Age (year)	51.16±6.82	48.52±9.67	0.271
BMI (kg/m^2)	32.96±0.99	30.23±1.20	0.087
FBG (mg/dL)	192.76±13.28	98.00±2.35	<0.001
HbA1c (HPLC) (%)	9.24±0.38	5.74±0.06	<0.001
HbA1c (IFCC) (mmol/molHb)	77.44±4.15	39.28±0.68	<0.001
Total cholesterol (mg/dL)	204.52±10.10	189.84±9.98	0.306
LDL cholesterol (mg/dL)	116.28±8.25	113.44±7.96	0.805
HDL cholesterol (mg/dL)	50.24±2.93	45.36±2.48	0.209
Triglyceride (mg/dL)	183.92±16.00	156.76±14.90	0.220
Total cholesterol/HDL cholesterol	4.22±0.22	4.32±0.24	0.770
Urea (mg/dL)	28.28±1.34	30.67±1.70	0.277
Creatinine (mg/dL)	0.64±0.03	0.77±0.04	0.004
Total protein (g/dL)	7.59±0.10	7.45±0.13	0.390
Albumin (g/dL)	4.34±0.07	4.47±0.06	0.170
AST (U/L)	26.92±4.77	24.96±2.48	0.718
ALT (U/L)	32.16±7.20	24.84±3.18	0.359
ALP (U/L)	93.06±7.98	77.17±3.80	0.085
GGT (U/L)	44.56±6.65	28.11±2.68	0.020
Folate (ng/mL)	10.19±1.36	10.97±1.43	0.699
Vitamin B12 (pg/mL)	264.11±57.36	220.38±18.69	0.486
25-hydroxy vitamin D (ng/mL)	12.18±3.94	15.32±2.32	0.521
WBC (10^3 uL)	8.67±0.31	7.13±0.36	0.002
RBC (10^{-6} uL)	4.91±0.11	4.81±0.11	0.523
HGB (g/dL)	13.07±0.40	13.70±0.42	0.282
HCT (%)	38.99±0.79	40.23±1.03	0.343
PLT (10^3/uL)	290.55±13.31	258.79±8.22	0.051
Uric acid (mg/dL)	5.43±0.37	5.53±0.26	0.820
Sediment 1 h (mm/saat)	30.20±7.01	15.58±3.13	0.108
Sodium (mEq/L)	139.10±0.36	140.61±0.38	0.006
Potassium (mEq/L)	4.68±0.08	4.60±0.09	0.517
Calcium (mg/dL)	9.66±0.08	9.53±0.07	0.248
eGFR (mL/dk/1.73)	104.12±2.23	100.80±2.62	0.340
CRP (mg/L)	7.49±1.36	3.07±0.55	0.002
Diabetes duration (years)	8.92±0.90	-	-
Smoking, yes (%)	4.5	36	0.012
Hypertension, yes (%)	54.5	0	<0.001
Family history, yes (%)	73.9	45.8	0.050
Low HDL, yes (%)	20	36	0.208
Hypercholesterolemia, yes (%)	52	40	0.395

Statistical evaluation by Chi-square, Fisher's exact, and student's *t*-tests. The results are shown as mean±SEM. *n*=Number of individuals; BMI=Body mass index; FBG=Fasting blood glucose; HPLC=High-performance liquid chromatography; IFCC=International Clinical Chemistry and Laboratory Medical Federation; HbA1c=Glycated hemoglobin; LDL=Low-density lipoprotein; HDL=High-density lipoprotein; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=Alkaline phosphatase; GGT=Gamma-glutamyltransferase; WBC=White blood cell; RBC=Red blood cell; HGB=Hemoglobin; HCT=Hematocrit; PLT=Platelet; eGFR=Glomerular filtration rate; CRP=C-reactive protein; SEM=Standard error of mean

Table 2: Results of Pearson's correlation between expression level of sirtuin 1 and other parameters

Variable	Patients (*n*=25)		Controls (*n*=25)	
	r	*P*	*r*	*P*
miR-181a	−0.558	0.005	0.115	0.586
miR-132	−0.435	0.034	−0.114	0.589
miR-9	−0.381	0.066	−0.136	0.517
miR-34a	−0.214	0.315	−0.320	0.118
Age (year)	−0.163	0.447	−0.073	0.727
FBG (mg/dL)	−0.125	0.562	−0.414	0.040
HbA1c (mmol/molHb)	−0.362	0.082	−0.234	0.261
Total cholesterol (mg/dL)	−0.243	0.253	−0.074	0.725
LDL cholesterol (mg/dL)	−0.186	0.385	−0.010	0.962
HDL cholesterol (mg/dL)	−0.044	0.840	−0.099	0.636
Triglyceride (mg/dL)	−0.250	0.239	−0.144	0.494
Total cholesterol/HDL cholesterol	−0.162	0.450	0.000	1.000
Urea (mg/dL)	0.093	0.666	0.134	0.524
Creatinine (mg/dL)	0.041	0.850	−0.207	0.322
Albumin (g/dL)	0.358	0.158	−0.007	0.979
Total protein (g/dL)	0.080	0.838	−0.045	0.884
AST (U/L)	−0.049	0.823	−0.105	0.618
ALT (U/L)	−0.034	0.873	−0.014	0.948
ALP (U/L)	−0.279	0.278	0.074	0.769
GGT (U/L)	−0.184	0.495	−0.050	0.840
BMI (kg/m^2)	−0.096	0.655	0.160	0.455

Statistical evaluation by Pearson's correlation. *n*=Number of individuals; FBG=Fasting blood glucose; HbA1c=Glycated hemoglobin; LDL=Low-density lipoprotein; HDL=High-density lipoprotein; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=Alkaline phosphatase; GGT=Gamma-glutamyltransferase; BMI=Body mass index

and miR-132 (r = −0.435, P = 0.034) in patients and FBG (r = −0.414, P = 0.040) in controls as well. However, there was no significant correlation between SIRT1 and other parameters (P > 0.05).

Pearson's correlation test showed that there was a significant positive correlation between miR-181a with miR-132 (r = 0.826, P < 0.001), miR-9 (r = 0.434, P = 0.030), miR-34a (r = 0.603, P = 0.001), and triglyceride (r = 0.494, P = 0.012) and also miR-132 with miR-9 (r = 0.412, P = 0.041), miR-34a (r = 0.792, P < 0.001), and HbA1c (r = 0.443, P = 0.027) in patients. There was a negative correlation between miR-181a with creatinine (r = −0.489, P = 0.013) and a positive correlation between miR-132 with miR-34a (r = 0.688, P < 0.001) and miR-181a (r = 0.565, P = 0.003) in control group. In addition, it was found that miR-34a was positively correlated with AST (r = 0.631, P = 0.001), ALT (r = 0.698, P < 0.001), and GGT (r = 0.605, P = 0.013) in patients and also AST (r = 0.550, P = 0.004) and ALT (r = 0.434, P = 0.030) in controls.

DISCUSSION

In recent years, T2DM and understanding its molecular mechanisms have gained great importance worldwide.

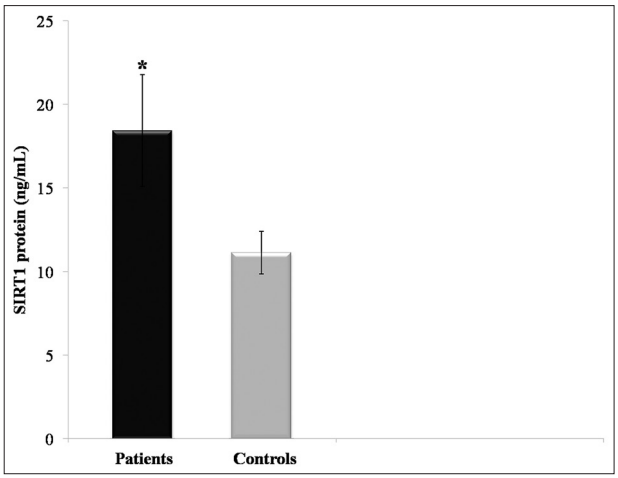

Figure 2: Sirtuin 1 protein levels in patient and control groups. Statistical evaluation by Student's *t*-test. The results are shown as mean ± standard error of mean. *Significant difference between patients and controls (P = 0.044)

The circulating miRNAs and related proteins have strong potential as novel biomarkers for early diagnosis and pathogenesis of various metabolic diseases such as T2DM. In literature, some miRNAs have been reported to be abundant and stable in serum and also potentially disease specific by targeting SIRT1. However, there are limited data worldwide and no such study in Turkey related to the effects of SIRT1 and miRNAs involved in SIRT1 regulation in T2DM. Therefore, in the present study, we performed our experiments on SIRT1 protein and related miRNAs.

Recently, a number of studies have been carried out on the potential use of serum SIRT1 and related miRNAs as biomarkers for elucidating the molecular mechanism of T2DM. In a previous study, researchers determined that 7 miRNAs including miR-9, miR-29a, miR-30d, miR34a, miR-124a, miR146a, and miR375 (P < 0.05) were significantly upregulated in T2DM patients compared to normal glucose levels individuals.[15] In another previous study, Liu *et al*. showed that expression levels of miR-34a (P < 0.05) and miR-34c (P < 0.01) were significantly higher in the T2DM group. Thus, these miRNAs had a potency to be used as biomarkers for T2DM diagnosis.[16] Zhou *et al*. observed that the level of serum miR-181a was significantly increased in patients (P < 0.01). They reported that miR-181a regulated SIRT1 and improved hepatic insulin sensitivity. Therefore, that miR-181a inhibition may be a potential new strategy for insulin resistance and T2DM treatment.[17] However, in the current study, no statistically significant difference was found for expression levels of miR-181a, miR-132, miR-9, and miR-34a between the groups (P > 0.05).

According to a study performed by Shao *et al*., SIRT1 levels were significantly decreased (P < 0.01) and miR-217 levels were significantly increased in the serum of T2DM

patients ($P < 0.01$). miR-217 was negatively correlated with SIRT1 ($P = 0.002$). Moreover, there was a significant association between Ln (albumin/creatinine ratio) with SIRT1 and miR-217 ($P < 0.05$).[18] In another study by Fathy *et al.*, serum SIRT1 levels were significantly lower in the normoalbuminuric group ($P < 0.05$). SIRT1 demonstrated a positive correlation with FBG in normoalbuminuric patients ($P < 0.05$) and also a negative correlation with microalbumin in macroalbuminuric patients ($P < 0.05$).[19] Collectively, these investigations suggest that serum miR-217 may be involved in the development of diabetic kidney disease by promoting chronic inflammation, renal fibrosis, and angiogenesis, and serum SIRT1 might be associated with minimal renal insufficiency in patients with type 2 diabetic nephropathy. However, these results are in controversy with our results in that SIRT1 protein level was significantly increased in patients with T2DM compared to controls ($P = 0.044$). Furthermore, we determined a negative correlation between SIRT1 protein with miR-181a ($P = 0.005$) and miR-132 ($P = 0.034$) in the patient group. This may be due to differences in the development of T2DM among populations and epigenetic regulation.

CONCLUSIONS

Our findings suggest that the increase in the SIRT1 protein expression in patients with T2DM may be considered as a compensatory mechanism in the body. miR-181a and miR-132 may be involved in the development of T2DM by targeting SIRT1. Therefore, SIRT1 has the potential to be a new noninvasive biomarker for T2DM. This is the first study to investigate the effects of SIRT1 and miRNAs involved in SIRT1 regulation in Turkish T2DM patients. Further research is needed to better understand the association between SIRT1 and T2DM in large cases.

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Conflicts of interest

There are no conflicts of interest.

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