The expression level of microRNA-122 in patients with type 2 diabetes mellitus in correlation with risk and severity of coronary artery disease

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Background
Type 2 diabetes mellitus (T2DM) has reached epidemic proportions worldwide. Coronary artery disease (CAD) is one of the most important causes of mortality worldwide. MicroRNAs (miRNAs) modulate gene expression and is involved in the pathogenesis of T2DM and CAD. The objective of the current study was to explore the expression pattern of miR-122 in T2DM with or without CAD. Moreover, we aimed to evaluate the association between miR-122 and risk and severity of CAD in T2DM.

Participants and methods
This cross-sectional controlled study enrolled 130 patients with T2DM and 110 control group. The enrolled diabetic patients were classified into two groups: seventy patients without CAD and 60 patients without CAD. All patients were investigated using a 12-lead standard ECG, echocardiography, and coronary arteriography. The serum MiR-122 expression profile was measured using quantitative real-time (qRT) PCR.

Results
miRNA-122 expression levels were significantly higher in T2DM, in particular patients with T2DM with CAD, compared with the control group. Interestingly, miRNA-122 expression levels were positively correlated with cardiometabolic risks and severity of coronary occlusion. Linear regression analysis test showed that miRNA-122 were independently correlated with high-density lipoprotein, ejection fraction, and uric acid. The power of miRNA-122 expression level to diagnose T2DM among studied participants was evaluated using receiver operating characteristic. The area under curve was 0.997 (95% confidence interval=0.993–1.00), with sensitivity of 96.9% and specificity of 99%, and regarding the power for differentiating patients with T2DM with CAD from patients with T2DM without CAD, the area under curve was 0.832 (95% confidence interval=0.763–0.902), with sensitivity of 93.3% and specificity of 96.86%.

Conclusion
The miRNA-122 expression levels were higher in the T2DM group compared with controls, in particular patients with CAD. The higher levels of miR-122 expression were strongly correlated with cardiometabolic risk factors and severity of coronary occlusion. Therefore, miR-122 expression levels seem to be a noninvasive biomarker for CAD.

Introduction
A preponderance of evidence suggests that diabetes mellitus (DM) has reached epidemic proportions worldwide, and its prevalence is rising [1,2]. In addition, DM augments the risk for coronary artery disease (CAD) by 2 to 6 folds [3], with mortality arising mainly from acute thrombotic cardiovascular events [4].

MicroRNAs (miRNAs) are a class of endogenous, noncoding, single-stranded short RNAs that negatively regulate gene expression at the post-transcriptional level by binding to the 3′ untranslated regions of target mRNAs [5]. Compelling evidence confirmed that alterations in the expression of microRNA contribute to the pathogenesis of diabetes. Profiling of microRNA expression in patients with diabetes has identified signatures associated with diagnosis, progression, prognosis, and response to treatment [6]. Accumulating data observed the crucial roles of miRNAs, particularly miR-122, in dyslipidemia and CAD pathogenesis [7].

Type 2 diabetes mellitus (T2DM) is a major independent risk factor for cardiovascular disease (CVD), the most common cause of morbidity and mortality among diabetic patients. The increasing incidence of diabetes and CVD places a huge burden on Egypt's healthcare resources. Thus, the objective of the current study was designed to explore the expression pattern of miR-122 in T2DM with or without CAD. Moreover, we aimed to evaluate the association between miR-122 and the risk and severity of CAD in T2DM.

Participants and methods
Participants
This study included 240 unrelated participants: 130 patients with T2DM and 110 healthy controls, who were matched with cases with respect to sex and ethnic origin. The diagnosis of diabetes was done according to ADA 2017; the enrolled diabetic patients were classified into two groups: seventy patients without CAD and 60 patients without CAD. The patients with type 1 diabetes mellitus (T1DM), patients with eGFR less than $60\,ml/min/1.73\,m^2$, patients with decompensated liver disease, patients with rheumatic valvular heart diseases, patients with decompensated heart failure, patients with previous myocardial infarction, or patients with recent cerebrovascular events (such as brain infarction or hemorrhage) within the past 6 months were excluded from this study. All patients were subjected to thorough history taking and full clinical assessment including blood pressure and anthropometric variables. BMI was calculated as weight in $kg/height$ in (m^2). The use of current antidiabetic and cardiac medications was determined through chart review and the standardized interview. The patients underwent elective coronary angiography for suspected CAD in the Cardiology Department of Zagazig University Hospitals. All patients were investigated using a 12-lead standard ECG and echocardiography. Coronary arteriography was performed for all patients by the Judkins technique for assessment of the lesions distribution and description and assessment of the severity of atherosclerotic CAD by using SYNTAX score, which is based on the baseline diagnostic angiogram. The total SYNTAX score was calculated from the summation of the individual scorings for each separate lesion by using a SYNTAX score algorithm available on the SYNTAX website (http://www.syntaxscore.com). CAD was considered significant if there was greater than or equal to 50% diameter stenosis in greater than or equal to one coronary artery. The Ethical Committee of the Faculty of Medicine, Zagazig University, approved our study protocol, and all participants signed a written informed consent form.

Collocation of blood and biochemical analysis
Venous blood samples were collected from participants after overnight fasting for determination of fasting C-peptide, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). Moreover, fasting and postprandial blood glucose level, HbA1c, and blood pressure were measured for all participants. HbA1c was measured in venous EDTA whole blood by the colorimetric method according to Trivelli and colleagues. Fasting serum insulin concentrations (FSI) were measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit provided by Biosource Europe S.A., Nivelles, Belgium. Homeostasis model assessments of insulin resistance (HOMA-IR) was calculated) was calculated as follow: [FSI (mU/ml)×fasting plasma glucose (FPG) (mg/dl)/405]. Serum TC, HDL-C, and TG concentrations were determined calorimetrically by kits purchased from Stanbio Laboratory (Texas, San Antonio, USA). Serum LDL-C was calculated according to the Friedewald formula. C-reactive protein (CRP) was measured using immunoturbidimetric assay on Roche/Hitachi cobas system (c501) autoanalyzer (Roche Diagnostics, Mannheim, Germany).

MiRNA extraction from sera
Total RNA was extracted from sera using miRNEasy RNA isolation kit (miRNeasy Mini Kit, Cat# 217004; QIAGEN, GmBH, Germany) according to the manufacturer's instructions. Total RNA was eluted by 30 µl of ribonuclease-free water. The RNA quality was then determined using UV/visible spectrophotometer. The ratio of absorbance at 260 and 280 nm was used to assess the purity of RNA. The quality of total RNA was detected by A260 to A280 ratio and 1.2% agarose gel electrophoresis.

Real-time PCR for the detection of serum miRNA-122 expressions levels
One microgram miRNA was used in reverse transcription with a miScript II RT Kit (Qiagen/SABiosciences Corporation, Frederick, Maryland, USA). Then, the cDNA was kept in −80°C till the real time-polymerase chain reaction (RTPCR) analyses.

Quantitative real-time-polymerase chain reaction (qRT-PCR) was carried out by using the Applied Biosystems 7500 fast PCR system (Applied Biosystems, Foster City, California, USA); 2×QuantiTect SYBR Green PCR Master Mix was used, 10×miScript Universal Primer, and 10×miScript Primer Assay, which was two types: one that targets the gene of interest (mature miRNA-122: hsa-miR-122-3p, Cat. No. MS00008428), MIMAT000 4590: 5'AACGCCAUUAUCACACUAAAUA 3'

(Qiagen), and the reference or housekeeping gene primer (RNU6 Human miRNA) (Qiagen). Cycler was set for 40 cycles. Initial activation step was carried out for 15 min at 95°C. Three-step cycle protocol was used: denaturation for 15 s at 94°C, annealing for 30 s at 55°C, and extension for 30 s at 70°C.

The miRNA-specific primers of miRNA-122 were chosen based on the miRNA sequences obtained from the miRbase database (http://microrna.sange r.ac.uk/). The relative gene expressions (fold change) of serum microRNA expressions levels were analyzed using the comparative threshold cycles (Ct) method [8].

Statistical analysis

Categorical variables were presented as frequencies and percentages and compared using the χ^2-test. Shapiro–Wilk's testing was conducted to determine the normal distribution of quantitative variables. Variables with normal distribution were compared using Student's *t*-test. Non-normally distributed variables were tested using the Mann–Whitney *U*-test. Correlations between continuous variables were determined by Pearson's correlation test. Logistic regression was conducted to evaluate the association between miRNA-122 expression levels and other correlated variables. Linear regression analysis tested the influence of the main independent variables against miRNA-122. Receiver operating characteristic (ROC) analysis was performed to assess the potential diagnostic accuracy of miRNA-122, the area under the curve (AUC), and the cutoff values. We considered *P* to be significant at less than 0.05 with a 95% confidence interval (CI).

Results

Among the studied participants, in the control group, 69.6% were male and 30.4% were female, and their mean age was 46.44±5.56 years. In the diabetic group, 64.3% were male and 35.7% were female, and their mean age was 47.11±5.3 years. Control and T2DM groups were matched for age, ethnicity, and sex.

Among T2DM groups, patients with T2DM without CAD comprised 67.8% male and 32.2% female, and their mean age was a 47.43±8.08 year. In patients with T2DM with CAD, 71.4% were male and 28.6% were female, and their mean age was 46.02±8.53 years. Both diabetic groups were matched for age, sex, and BMI.

Anthropometric and laboratory characteristics of the studied participants are summarized.

As expected, diabetic patients had significantly higher values of systolic blood pressure (SBP) as well as lipid

Table 1 Clinical, anthropometric, and laboratory characteristics of the studied groups

Variables	Control group (mean±SD) (*N*=110)	T2DM group (mean±SD) (*N*=130)	*P* value
Systolic blood pressure (mmHg)	122.9±3.58	131.06±16.87	<0.001
Diastolic blood pressure (mmHg)	85.7±5.27	87.7±10.92	0.184
Duration of DM (years)	–	12.4±5.39	–
Family history of CAD [*n* (%)]	–	42 (32.2)	–
BMI (kg/m^2)	32.8±5.018	33.7±5.43	0.278
Waist/hip ratio	1.2±0.186	1.35±0.198	0.072
Fasting plasma glucose (mg/dl)	87.68±4.26	181.3±30.1	<0.001
FSI (IU/ml)	7.54±2.208	16.67±8.12	<0.001
HOMA-IR	1.64±0.507	6.71±2.663	<0.001
HbA1c (%)	4.8±0.23	9.63±1.762	<0.001
HDL cholesterol (mg/dl)	55.86±7.34	36.09±6.63	<0.001
LDL cholesterol (mg/dl)	83.8±22.03	139.9±38.56	<0.001
Total cholesterol (mg/dl)	128.4±40.57	172.6±36.7	<0.001
Triglycerides (mg/dl)	130.4±12.42	163.76±31.12	<0.001
Uric acid (mg/dl)	4.6±0.514	7.3±2.276	<0.001
hs-CRP (µg/ml)	2.12±0.514	6.80±4.27	<0.001
microRNA-122	1.19±0.25	2.5±0.61	<0.001

CAD, coronary artery disease; FSI, fasting serum insulin; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessments of insulin resistance; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus. *P<0.05 when compared with control group.

profile (TG, TC, and LDL) compared with control group (Table 1). Furthermore, diabetic patients had significantly higher values of FPG, FSI, HOMA-IR, and HbA1c compared with the control group. In addition, non-traditional risk factors such as serum uric acid and hs-CRP levels were significantly higher in diabetic patients compared with control group. On the contrary, patients with T2DM had significantly lower values of HDL compared with the control group (*P*<0.001).

Anthropometric and biochemical characteristics of the T2DM groups

Among patients with T2DM (*n*=130), 60 patients had CAD confirmed by coronary angiography, and they had a significantly positive family history of CAD and higher values of SBP, as well as diastolic blood pressure (DBP), compared with patients with T2DM without CAD. Furthermore, patients with T2DM with CAD had significantly higher values of TC, TG, FPG, HOMA-IR, and HbA1c compared with patients with T2DM without CAD. On the contrary, diabetic patients had significantly lower values of ejection fraction and HDL compared with the control group. Regarding cardiac and diabetic medication, there were significant differences between the two diabetic groups (*P*<0.001) (Table 2).

Table 2 Clinical, anthropometric, and laboratory characteristics of type 2 diabetes mellitus groups

	Without CAD (mean±SD) (n=70)	With CAD (mean±SD) (N=60)	P value
Family history of CAD [n (%)]	9 (12.8)	33 (55)	<0.001
Duration of DM (years)	12.4±5.22	12.5±5.63	0.929
Smoking	22 (31.4)	21 (35)	0.121
Systolic blood pressure (mmHg)	132.7±14.91	152.1±12.2	<0.001
Diastolic blood pressure (mmHg)	90.5±7.42	99.8±9.6	<0.001
BMI (kg/m^2)	33.8±3.95	33.7±6.79	0.890
Waist/hip ratio	1.34±0.17	1.3±0.22	0.390
Ejection fraction (%)	48.41±7.465	45.5±5.154	<0.05
Fasting plasma glucose (mg/dl)	170.7±34.2	194.5±17.2	<0.001
FSI (IU/ml)	15.84±9.65	17.63±5.424	0.206
HOMA-IR	6.68±4.472	8.35±2.26	<0.05
HbA1c (%)	9.44±1.89	10.8±0.95	<0.001
HDL cholesterol (mg/dl)	37.41±7.46	34.56±5.15	<0.05
LDL cholesterol (mg/dl)	137.5±41.14	142.7±35.45	0.451
Total cholesterol (mg/dl)	160.27±45.2	187.01±12.9	<0.001
Triglycerides (mg/dl)	148.2±32.3	181.8±16.7	<0.001
Uric acid (mg/dl)	5.03±1.926	7.95±2.95	<0.001
hs-CRP (μg/ml)	4.538±3.926	9.45±2.95	<0.001
DM treatment:			
Oral drugs	44	10	<0.001
Insulin	8	42	
mixed	18	8	
Cardiac medication			
β-blocker	11	51	<0.001
ACEI/ARB	23	48	
Statin	33	49	

CAD, coronary artery disease; FSI, fasting serum insulin; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessments of insulin resistance; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus. *P<0.05.

Comparison of miRNA-122 expression levels in different studied groups

Our results revealed statistically significant higher values of miRNA-122 expression levels in the T2DM group (2.5±0.61) compared with controls (Table 1). In an attempt to evaluate the association between the miRNA-122 signature profile and CAD, we subdivided the T2DM group into patients without and with CAD, and we found significantly higher values of miRNA-122 expression levels in T2DM with CAD (2.89±0.53) compared with patients with T2DM without CAD (2.24±0.51) (P<0.001) (Fig. 1a).

miRNA-122 expression levels in T2DM with CAD stratified according to the number of coronary arteries occlusion

Among patients with CAD (n=60), patients with multiple vessels occlusion (n=32) had statistically significant higher values of miRNA-122 expression levels (3.24±0.597) compared with patients with two-vessel occlusion (n=17, 2.59±0.465) and patients with single-vessel occlusion (n=11, 2.31 ±0.513) (P<0.001) (Fig. 1b).

The severity of CAD using syntax score

We estimated the severity of CAD according to coronary angiography findings, and we used a syntax score. The mean and standard deviation of syntax score in patients with CAD was 25.96±5.035. Sixteen patients had a low score (0–22), 19 patients had an intermediate score (23–32), and 25 had a high score (≥33) (Fig. 2a).

Correlation between miRNA-122 expressions levels and severity of CAD using the syntax score

In CAD subgroup (n=60), there were significant positive correlations between miRNA-122 expression levels and syntax score (P<0.001) (Fig. 2b).

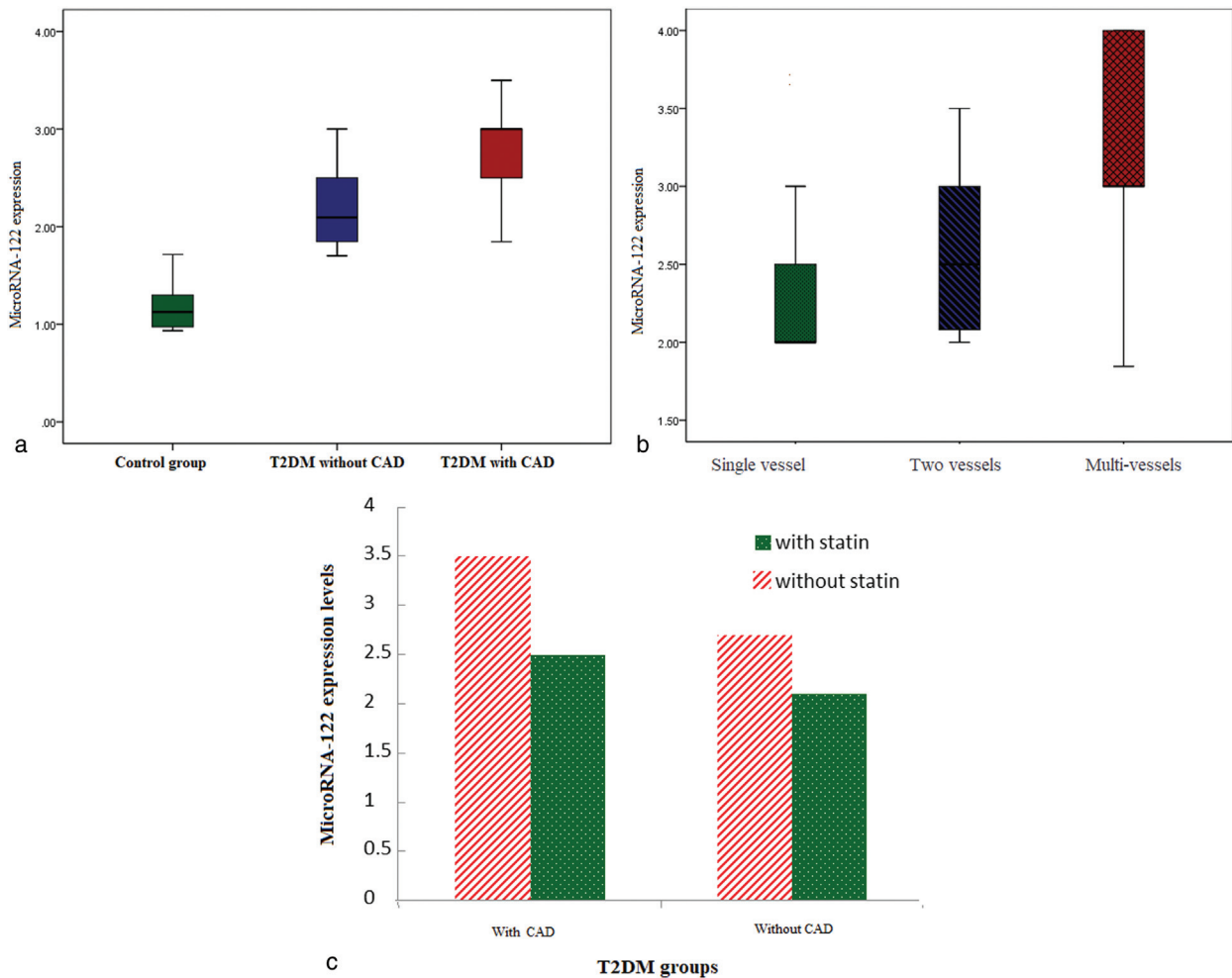### Effect of statin therapy on miRNA-122 expression levels

To better evaluate the relation between statin therapy and miRNA-122 expression levels in both T2DM groups, we subdivided each group to patients treated with statin (atorvastatin) and patients without statin therapy. Among patients with CAD (n=60), patients without statin therapy (n=11) had statistically significant higher values of miRNA-122 expression levels (3.5±0.597) compared with patients treated with statin therapy (n=49, 2.5±0.465). Interestingly, non-ischemic group (n=70) patients without statin therapy (n=11) had statistically significant higher values of miRNA-122 expression levels (2.7±0.597) compared with patients treated with statin therapy (n=49, 2.1±0.465) (P<0.001) (Fig. 1c).

Pearson correlation between miRNA-122 expression levels and clinical and laboratory parameters among patients with T2DM

Our results revealed that in the CAD group, there were significant positive correlations between miRNA-122 expression levels and DBP, BMI, TC, TG, LDL, HOMA-IR, HbA1c, hs-CRP, as well as uric acid. On the contrary, miRNA-122 expression levels were significantly negatively correlated with ejection fraction and HDL (P<0.05) (Table 3).

Regarding patients with T2DM without CAD, there were significant positive correlations between miRNA-122 expression levels and DBP, waist/hip ratio TC, TG, LDL, hs-CRP, as well as uric acid. On the contrary, miRNA-122 expression levels were significantly negatively correlated with HDL (P<0.05) (Table 3).

Fig. 1

(a) Comparison of microRNA-122 expression level in different studied groups; (b) comparison of microRNA-122 expression level in coronary artery disease group; (c) effect of statin therapy on serum microRNA-122 expression level in type 2 diabetes mellitus groups.

Linear regression analysis in T2DM groups

Our results found that HDL, ejection fraction, and uric acid were the main associated variables with miRNA-122 expression levels among other clinical and laboratory biomarkers ($P<0.05$) (Table 4).

Logistic regression analysis test used to evaluate the association of microRNA-122 with the severity of CAD among T2DM.

Our study revealed that after adjustment for age, sex, and BMI, the only variables associated with severity of CAD among T2DM were microRNA-122, with odd's ratio of 7.396 (CI=3.215–17.012), and FPG, with odd's ratio of 1.032 (CI=1.013–1.051) ($P<0.001$) (Table 5).

The accuracy of circulating miRNA-122 expression level in the diagnosis of CAD by receiver operating characteristic analysis

The power of miRNA-122 expression level to diagnose T2DM among studied participants was evaluated using ROC analysis. The AUC was 0.997 (95% CI=0.993–1.00) with sensitivity of 96.9%, specificity of 99%, and the cutoff value was 1.707 (Fig. 3a).

The accuracy of circulating miRNA-122 expression level for differentiating T2DM with CAD from T2DM patients without CAD by receiver operating characteristic analysis

The power of miRNA-122 expression level for differentiating patients with T2DM with CAD from patients with T2DM without CAD was evaluated using ROC analysis. The AUC was 0.832 (95% CI=0.763–0.902) with sensitivity of 93.3% and specificity of 96.86%, and the cutoff value was 2.442 (Fig. 3b).
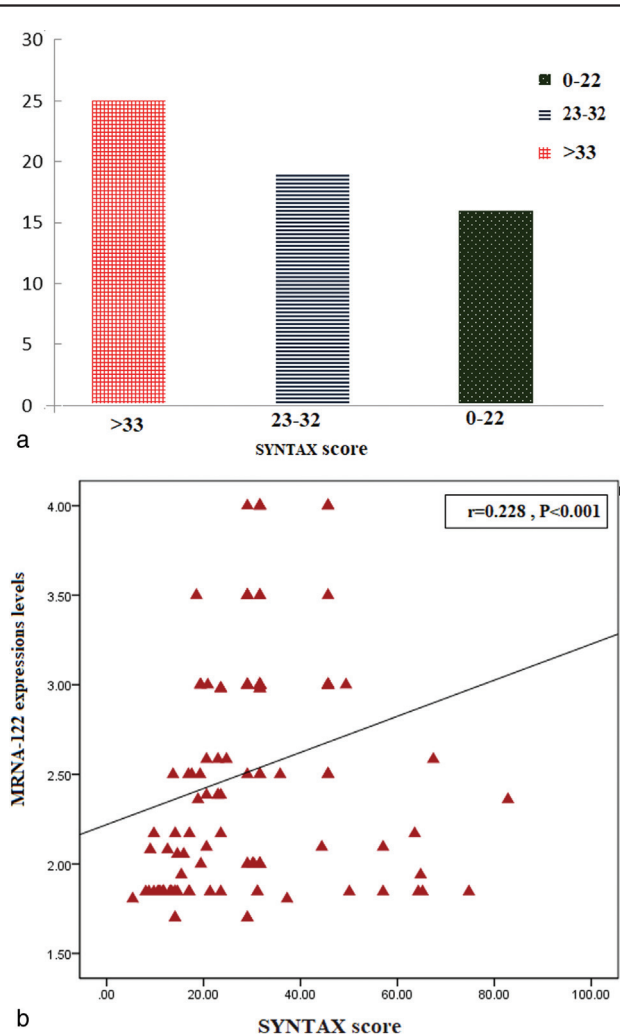
Discussion

T2DM is a major independent risk factor for CAD, and the most common cause of morbidity and mortality among diabetic patients [9]. Substantial evidence implicates miRNAs as a critical mediator in the pathophysiology of CADs such as endothelial

dysfunction, inflammation, apoptosis, angiogenesis, atherosclerosis, and neointimal hyperplasia or restenosis [10]. Recently published studies highlighted the value of miR-122 in fatty acid synthesis and oxidation through several key genes involved in lipid metabolism [7].

Fig. 2

a

b

(a) SYNTAX score of the coronary artery disease group; (b) correlation between SYNTAX score and microRNA-122 expression level of the coronary artery disease group.

In fact, the better understanding of the pathogenesis of CVD and T2DM is of great interest for early prediction and identification of at-risk patients and for better clinical management. Therefore, the aim of the current study was to explore the expression pattern of miR-122 in T2DM with or without CAD. Moreover, we aimed to evaluate the association between miR-122 and the risk and severity of CAD in T2DM.

The results presented here are innovative, as this study was the first Egyptian study that investigated the possible association of miRNA-122 expression levels in the T2DM group as well T2DM group with CAD. Our results revealed statistically significant higher values of miRNA-122 expression levels in the T2DM group compared with controls.

Table 3 Correlation between microRNA-122 expression levels and cardio metabolic risk factors among type 2 diabetes mellitus

Variables	Without CAD (mean±SD) (n=70)		With CAD (mean±SD) (N=60)	
	r	P	r	P
SBP (mmHg)	0.183	0.163	0.351	<0.001
DBP (mmHg)	0.342	<0.001	0.421	<0.001
BMI (kg/m^2)	0.394	<0.001	0.129	0.286
Waist/hip ratio	0.157	0.231	0.497	<0.001
Ejection fraction (%)	−0.271	<0.001	−0.015	0.894
TC (mg/dl)	0.386	<0.001	0.344	<0.001
TG (mg/dl)	0.314	<0.001	0.434	<0.001
LDL-c (mg/dl)	0.082	<0.001	0.651	<0.001
HDL-c (mg/dl)	−0.271	<0.05	−0.417	<0.001
FPG (mg/dL)	0.221	0.090	0.016	0.895
FSI (IU/ml)	0.368	<0.001	0.041	0.735
HOMA-IR	0.337	<0.001	0.031	0.796
HbA1c (%)	0.221	0.090	0.016	0.895
hs-CRP (µg/ml)	0.265	<0.05	0.693	<0.001
Uric acid (mg/dl)	0.253	<0.05	0.684	<0.001

DBP, diastolic blood pressure; FPG, fasting plasma glucose; FSI, fasting serum insulin; HOMA-IR, homeostasis model assessments of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure. *P<0.05, statistically significant.

Table 4 Linear regression analysis test used to explore the influence of the main independent variables against microRNA-122 (dependent variable) in patients with type 2 diabetes mellitus

Model	Unstandardized coefficients		Standardized coefficients	t	P	95% CI	
	B	SE	β			Lower bound	Upper bound
(Constant)	0.521	0.284		1.835	0.068	0–0.039	1.081
Waist/hip ratio	0.133	0.147	0.032	0.903	0.368	−0.157	0.423
HOMA-IR	0.008	0.008	0.041	0.979	0.329	0–0.008	0.025
HDL-c	−0.079	0.008	−1.103	9.706	<0.001	−0.095	−0.063
Ejection fraction (%)	0.077	0.010	0.775	7.712	<0.001	0.058	0.097
Uric acid	0.106	0.008	0.539	12.78	<0.001	0.090	0.123

CI, confidence interval; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessments of insulin resistance. *P<0.05.

Table 5 Logistic regression analysis test used to evaluate the association of microRNA-122 with severity of coronary artery disease among patients with type 2 diabetes mellitus.

	B	SE	t	P value	Odd's	95% CI	
						Lower	Upper
MicroRNA-122	2.001	0.425	22.163	<0.001	7.396	3.215	17.012
BMI	−0.037	0.041	0.824	0.364	.964	0.890	1.044
FPG	0.031	0.009	10.799	<0.001	1.032	1.013	1.051
Constant	−9.644	2.448	15.526	0.000	0.000		

CI, confidence interval; FPG, fasting plasma glucose.

Fig. 3
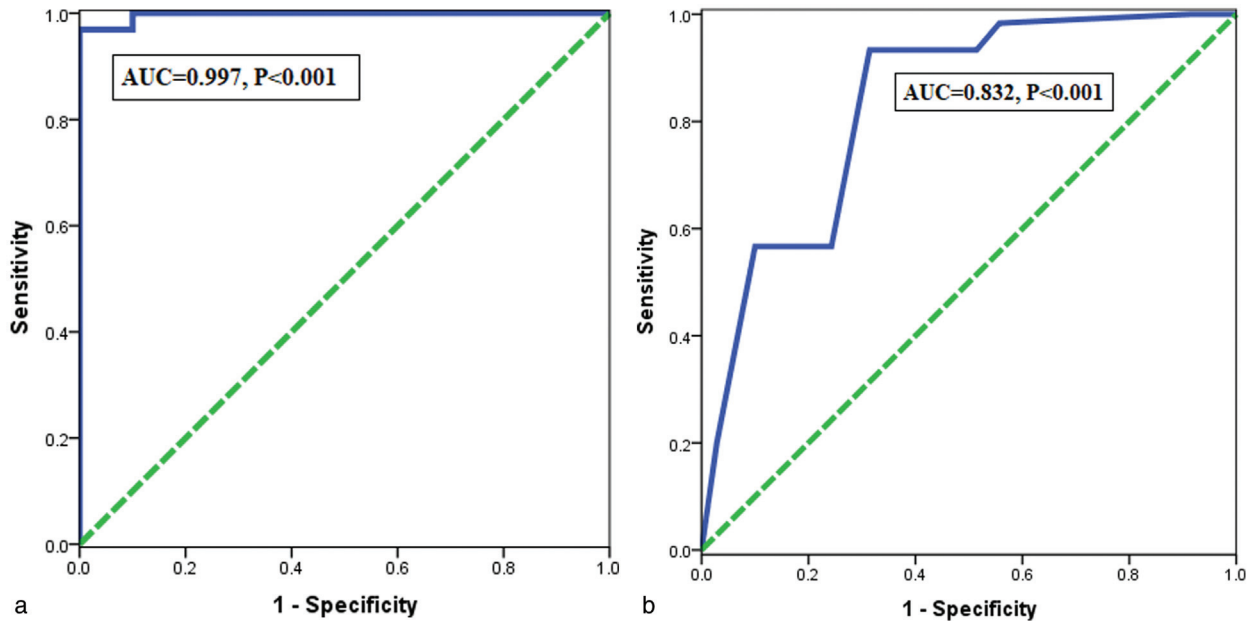
(a) Receiver operating characteristic curve for microRNA-122 as diagnostic biomarkers for diagnosis of type 2 diabetes mellitus among studied participants; (b) receiver operating characteristic curve for microRNA-122 as diagnostic biomarkers for diagnosis of coronary artery disease among type 2 diabetes mellitus.

Similar to our result, a study conducted by Willeit *et al.* [11] found high circulating miR-122 levels in patients with metabolic syndrome and T2DM.

Our results revealed that there were significant positive correlations between miRNA-122 expression levels and DBP, BMI, TC,TG, LDL, HOMA-IR, HbA1c, hs-CRP, as well as uric acid. In addition, miRNA-122 expression levels and FPG were independently correlated with CAD after adjustment of other cofactors, and HDL, ejection fraction, and uric acid were the main associated variables of miRNA-122 expression levels among other clinical and laboratory biomarkers.

Our findings are in concordance with the study by Gao *et al.* [7], in which the levels of miR-122 were positively correlated with TC, TG, and LDL-C levels in both hyperlipidemia patients and controls. Multiple logistic regression analysis demonstrated that the increased

levels of miR-122 were associated with CAD presence, even after adjustment for other cardiovascular risk factors.

Many recent studies have proposed that miR-122 is primarily expressed in the liver, and regulates the expression of various genes associated with cholesterol and fatty acid metabolism [12]. Even more importantly, experimental studies found that inhibition of miR-122 led to markedly lower plasma cholesterol levels, inhibited hepatic lipid synthesis, and enhanced hepatic fatty acid oxidation [13–16].

According to our study among patients with T2DM, approximately 81.6% of patients with T2DM with CAD were treated with statin and 47.1% of patients with T2DM without CAD treated with statin. To better evaluate the relation between statin therapy and miRNA-122 expression levels in both T2DM groups, we subdivided each group to patients treated with

statin and patients without statin therapy. Interestingly, we found that both ischemic and non-ischemic diabetic groups treated with statin had significantly lower miRNA-122 expression levels compared with other patients not treated with statin. Similar to our results, the Anglo-Scandinavian Cardiac Outcomes Trial found that after 12 months of atorvastatin therapy, the circulating miR-122 level reduced compared with the baseline levels [17].

To better elucidate the association between miRNA-122 expression profile and CAD, we subdivided T2DM group into patients without and with CAD, and we found significantly higher values of miRNA-122 expression levels in T2DM with CAD compared with patients with T2DM without CAD. Interestingly, there were significant positive correlations between miRNA-122 expression levels and severity of coronary artery occlusion by syntax score.

In agreement with our results, a study conducted by Gao et al. [7] revealed that levels of miR-122 were significantly increased in patients with hyperlipidemia compared with controls. In addition, they found that miR-122 was positively correlated with the severity of CAD quantified by the Gensini score [7]

There are intriguing reports suggesting that expression levels of miR-122 are increased in infarcted areas as well as in border areas after acute myocardial infarction [17].

On the contrary, a study by D'Alessandra et al. [18] found that plasma levels of miR-122 were lower in patients with AMI than healthy controls. Moreover, in contrast to our findings, a study by Wang et al. [19] detected that there was a non-significant difference of plasma miR-122 levels between patients with AMI and healthy participant.

The diverse results summarized above may owing to the difference of the studied population, as our studied groups had chronic but not acute ischemia.

For further evaluation of our results, we tested our findings by using ROC test, which revealed that diagnostic power of miRNA-122 expression levels in differentiating T2DM from control was stronger than that differentiating CAD from the nonischemic group.

Conclusion
Our results revealed statistically significant higher values of miRNA-122 expression levels in the T2DM group compared with controls in particular patients with CAD. The higher levels of miR-122 expression levels were strongly correlated with cardiometabolic risk factors and severity of coronary occlusion. Therefore, miR-122 expression levels seem to be a noninvasive biomarker of the CAD in patients with T2DM.

Conflicts of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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