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Association of Circular RNA 0054633 Expression Level with Cardiovascular Risk Factors in Patients with or Without Type 2 Diabetes Mellitus

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Abstract
Background: Circular ribonucleic acids (circRNAs) as a member of long non coding RNAs have a chief role in gene regulation and expression of proteins,lackingof the terminating 5'-cap and 3'-polyadenylated tail structures providecircRNAs with greater biological stability than linear RNAs. Among many tissues,circRNAsexpression levelsare ten times higher than other RNA types.Additionally, recent studies haveshown that circRNAs also have a major role in diabetes mellitus (DM) pathogenesis and itsrelated complications.We targetedto detect the association of circular RNA 0054633 expression level with cardiovascular risk factors in patients with or without type 2 diabetes mellitus (T2DM), and to examine itsrole as a biomarker for early diagnosis of pre-diabetes and T2DM.
Patients and Methods:A whole number of 108 individuals classified into three groups: group (1) was 36 healthy subjects, group (2) were 36pre-diabetics and group (3) including 36 T2DM patients. Real- time polymerase chain reaction (RT-PCR) was done for estimation of bloodcircRNA 0054633expression level.
Results: The currentresearch revealedsignificant risein CircRNA 0054633 expression level in type 2 diabetes mellitus (T2DM) group compared to control and the pre-diabetes groups ($p<0.05$).ROC analysis revealed that the area under the curve (AUC) of CircRNA 0054633 level in blood of pre-diabetics and diabetic patients was (0.951,0.83) with specificity of (97.2%, 94.4%) respectively and sensitivity of (94.4%, 86.1%)respectivelyat cut-off point(1.63, 3.94) indicatingCircRNA 0054633as a good predictor marker of pre-diabetes and T2DM.Linear regression analysisrevealed thatthe increase in HbA1c was independently related to the elevated plasma CircRNA 0054633 expression level.
Conclusions: BloodCircRNA 0054633expression level may represent a sensitive predictor for diagnosis of pre-diabetes and type 2 DM as there is a still need for novel biomarkers for early diagnosis of them.
Key words:T2DM, Pre-diabetes, CircRNA, Real time PCR, Expression

Introduction
Diabetes mellitus (DM), one of the major health challenges nowadays is a metabolic disease which manifested by hyperglycemia as a result of deficiency in insulin release, insulin exploit or both [1]. Regarding to the World Health Organization, the amount of cases with DM has rapidly augmented in the preceding decade all over the world. DM prevalence elevated from 108 million (4.7%) in 1980 to 425 million (8.5%) in 2017, and it is expected to reach 629 million in 2045 [2].In the developing countries, DM as a Health Care Providers challenge represents the fourth leading cause of death [3]. According to International Diabetes Federation (IDF), Egypt is considered one of the highest ten countries in the number of cases having DM worldwide. In Egypt, the prevalence of DM is estimated about 15.56% amongindividuals between 20 and 79 years of age, with ayearly death of 86,478 associatedwith diabetes [4]. Noteworthy, there is still a defect in DM diagnosis which affect about 193 million people all over the world who aren't aware of their illness, however the

alarming effect of its prevalence [5]. Multiple factors which participate in DM progression are divided into genetic and environmental influences as obesity, lack of exercise, stress and aging [6]. Impaired glucose tolerance (IGT) is defined as the interval between normal glucose tolerance and overt diabetes. Individuals with IGT are known to have "pre-diabetes" [7]. In Egypt, around 2.2 million individuals are estimated to have pre-diabetes according to IDF in 2013.Most cases with pre-diabetes and 43% of cases having DM are reported to be likely undiagnosed [4]. Non-coding RNAs (ncRNA) can be divided into two groups, housekeeper and regulatory ncRNAs which can be classified according to length into small non-coding RNAs (<200 bp), and long ncRNAs (lncRNAs) (>200 bp) [8]. The circRNAs as a member of lncRNAs are known as a unique type of RNAs with higher biological stability than linear RNAs as a result of lackingof the terminating 5'-cap and 3'-polyadenylated tail structures[9]. The wide distribution of CircRNAs in the cytoplasm and nucleus and also their presence in some body fluids such as saliva and serum facilitates their contribution in human biological functions [10].Many studies suggested the role of circRNAs as micro-RNA (miRNAs) sponges which control the up regulation and the downregulation of miRNAs target genes expression. Also, they act as RNA-binding proteins (RBPs) sponges helping in the post-transcriptional regulation of gene expression [11]. Newresearches have suggested the main role of circRNAs in the pathogenesis of T2DM and related vascular complications. In addition to their role in various biological processes as cell cycle and mitotic cell cycle arrest, they play important role in catabolism of molecules which is closely related to proliferation of β cells [12]. Large scales of studies have suggested the major effect of circRNAs non-regulation in multiple metabolic disorders like obesity, hypertension, and cardiovascular diseases [11]. The target of our research was to detect the association of circular RNA 0054633 expression level with cardiovascular risk factors in patients with or without T2DM, and to examine its role as diagnostic biomarker in early diagnosis of pre-diabetes and prediction of T2DM.

Subjects and Methods

The study was done in Medical Biochemistry and Internal Medicine Departments, Faculty of Medicine, Zagazig University. This study included 108 subjects aged between 48-60 years old: 36 patients with T2DM, 36 pre diabetic cases with impaired glucose tolerance, and 36 healthy individuals. Both Egyptian patients and controls are enlisted from outpatient clinics of the Endocrinology Unit of Internal Medicine Department; Zagazig University hospital. According to the 2017 The American Diabetes Association (ADA) (13) criteria for the diagnosis of diabetes ,patients were diagnosed T2DM if they have one of the nextstandards: (i) fasting plasma glucose (FPG) level of 126 mg/dL (7 mmol/L) or higher; fasting is defined as no caloric intake for at least 8 hours, (ii) a 2-hours plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT),or (iii) a random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycaemia (polyuria, polydipsia, polyphagia, weight loss) or hyperglycaemic crisis or (iiii) haemoglobin A1c (HbA1c) level of 6.5% or higher. Also, prediabetic patients could be diagnosed if they have one of the following criteria: (i) FPG 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9 mmol/l) [IFG], (ii) 2-h PG in the 75-g OGTT 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.0 mmol/l) [IGT] or (iii) a glycatedhaemoglobin (HbA1c%) was 5.7–6.4% (ADA,2017). All groups were non-smoker, normotensive, non-obese, uncomplicated, no history of coronary artery disease (CAD), liver and renal disease, cancer, autoimmune disorders, and inflammatory diseases and type-1 DM. All included groups were exposed to complete history taking, full clinical checkenclosing anthropometric measurements. Estimation of body mass index (BMI) was done by dividing body weight in kilograms by (height in square meters) (14). Laboratory investigations including fasting and 2 hours post prandial blood glucose

levels, HbA1c%, lipid profile [serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and calculation of low density lipoprotein cholesterol (LDL-c)].All participants signed an informed written consent before enrolment in the study and the study design was permitted by the Ethical Committee of Faculty of Medicine, Zagazig University.After an overnight fasting, 5 ml of intravenous blood were taken from every individual by sterile vein-puncture and separated into three samples: The first sample was 2ml of blood were collected in an EDTA containing tubes and were divided into two aliquots; for colorimetric determination of Glycated haemoglobin as percent of total haemoglobin using Stanbio Laboratory, Boerne, Texas, USA [15] and the reaming part was stored at -20 °Cfor total RNA extraction. The second sample was 1 ml blood into sodium fluoride tube, for measurement of FBG and 2 hours postprandial blood glucose by enzymatic colorimetric method using Spinreact kit,Girona,Spain (16).The third sample was 2ml of blood and transported into a plain tube, left to clot at 37°C, centrifuged for 15 minutes at 4000 r.p.m.Total cholesterol and triglyceride levels were measured by routine enzymatic methods (Spinreact, Girona, Spain)[17-18]. HDL cholesterol concentration was detected after precipitation of the apoB-containing lipoproteins[19]. The LDL cholesterol level was measured using the Friedewaldformula [20].

Real time PCR analysis for CircRNA (0054633) expression

Total RNA was taken out from whole blood samples using Total RNA Extraction Kit, Bio Flux, Simply P Total RNA Extraction Kit, and Chinaaccording to the manufacturer's instructions. The yield and purity of RNA were detected by Milton Roy UV spectrophotometer by determination of the a260/ a280 ratio. Reverse transcription step and formation of complementary DNA (cDNA) was done byHiSenScriptTMRH [-] for cDNA Synthesis Kit, INtRON Biotechnology, China. Each reaction was done on ice with a total volume of 20 µl, containing 1 µl of Enzyme Mix solution, 10 µl of 2 X RT Reaction solutions, 5µl of template RNA and 4 µl of nuclease free water. Incubation was done for 45 min at 42°C then, 10 min at 85°C to inactivate reverse transcriptase enzyme. The cDNA were kept at –20°C till real-time PCR step. Quantitative or real-time PCR was achieved by TOPreal^{TM}qPCR 2X PreMIX (SYBR Green with low ROX Kit). Real-time PCR was done to a total volume of 20 µl, containing 10 µl of SYBR green Master Mix; 4 µl of nuclease-free water, 4 µl of Template cDNA and 1 µl of each primer (forward & reverse). The following designed primers (Midland, Texas) were used: CircRNA (0054633):
Forward primer sequence:
5` TTGCTTTCTACACTTTCAGGTGAC3`, reverse primer 5`,
GCTTTTTGTCTGTAGTCAACCACCA`,
And
GAPDH forward primer sequence: 5` TGTTGCCATCAATGACCCCTT3`,
Reverse primer:5` CTCCACGACGTACTCAGCG3`.
The PCR condition for CircRNA (0054633) amplification contained three phases: initial activation phase at 95°C for 10 minutes followed by 45 cycles at 95°C for 10 sec; 60°C for 15sec; 72°C for 30 minute; and a final extension phase at 72°C for 10 minutes. Finally, fluorescence detection and data analysis were performed using Strata gene MIX3000P RT PCR. The relative quantification (RQ) of CircRNA (0054633) gene expression was done using comparative cycle threshold ($2^{-\Delta\Delta Ct}$ method) [21] where the amount of the target gene is normalized to an endogenous reference gene (GAPDH) and relative to a control.

Statistical analysis

Results were collected, tabulated and statistically analyzed by statistical package (SPSS version 22, Inc., Chicago, Illinois, USA).The description of data was expressed as mean

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value and standard deviation [SD] for quantitative data, frequency and percentage for qualitative date. F test and one-way analysis of variance (ANOVA) for comparison of two or more than two normally distributed quantitative variables, respectively. Linear regression analyses were done to test the effect of the main independent variables against blood circRNA 0054633 expression levels. Correlation analysis was performed using Person correlation method. Receiver operating characteristic (ROC) curve analysis was done to detect optimal cut-off values of CircRNA (0054633) with sensitivity and specificity for diagnosis of pre-diabetes and T2DM.

Results

Table (1): Descriptive statistics of the demographic parameters in the studied groups

Parameter	Group1 Control (N=36)	Group2 Pre-diabetes (N=36)	Group3 T2DM (N=36)	F. test	P value	P1	P2	P3
Age (mean ± SD, years)	54.1±3.2	54.6±3.3	54.7±3.4	0.30	0.74	0.5	0.44	0.89
Gender -Male -Female	 25(69.4%) 11(30.6%)	 26 (72.2%) 10 (27.8%)	 28(77.7%) 8 (22.3%)	X2 0.66	 0.719	 0.8	 0.42	 0.59
BMI(Kg/m^2)	23.52±0.64	23.8±0.61	23.9±0.63	5.48	0.05	0.06	0.0133	0.36
SBP (mm Hg)	113.5±8	117.5±7.1	118±6.5	4.24	0.02*	0.03*	0.01*	0.76
DBP (mm Hg)	75.8±5	76.3± 4.8	77.9±4.4	1.93	0.15	0.67	0.06	0.15

F test: one way ANOVA, X2: Chi square test, BMI= body mass index. SBP=systolic blood pressure. DBP=diastolic blood pressure.
*Significant P value (<0.05)
P1: Statistical significance of pre-diabetes when compared with control group.
P2: Statistical significance of T2DM when compared with control group.
P3: Statistical significance of T2DM when compared with pre-diabetes.

3.1. Demographic parameters in all studied groups

Regarding to demographic data, our results revealed that there were no significant statistical differences as regards age,gender, body mass index (BMI), and diastolic blood pressure. Noteworthy, there were significant differences of systolic blood pressure (SBP) between three studied groups by ANOVA (p=0.02). Deeper analysis by F test revealed significant increase of SBP in patients with pre-diabetescompared with control group (p=0.03), and in patients with T2DM when compared with control group (p=0.01), table (1).

Table (2): Biochemical characteristics of the studied groups

Parameter	Group1 ControlN=36	Group2 Pre-diabetesN=36	Group3 T2DMN=36	F test	Sig.	P1	P2	P3
-FPG(mg/dl)	90.6±6.3	120±3.28	222.5±33.5	440.98	<0.001*	<0.001*	<0.001*	<0.001*
-2hPPG(mg/dl)	93.9±7.3	162.5±10.5	266.5±23.7	1120.6	<0.001*	<0.001*	<0.001*	<0.001*
-HbA1c(%)	5±0.23	6±0.2	9±0.32	2237.1	<0.001*	<0.001*	<0.001*	<0.001*
-T.Cholest (mg/dl)	172.3±13.3	222.5±10.5	237.1±16.7	220.7	<0.001*	<0.001*	<0.001*	<0.001*
HDLc (mg/dl)	48.5±2.3	47.3±1.6	36±1.7	461.2	0.007	0.0123	<0.001*	<0.001*
LDLc (mg/dl)	101.4±7.1	153.5±5.3	167.5±5.2	1223.6	<0.001*	<0.001*	<0.001*	<0.001*
TG.(mg/dl)	111.5±42.3	108.2±34.3	167.6±65.8	16.48	<0.001*	0.775	<0.001*	<0.001*

F test: one way ANOVA. FPG= fasting plasma glucose, 2 hPPG= 2 hour post prandial glucose, HbA1c= Glycated hemoglobin, HDL-c = high density lipoprotein cholesterol, T.Cholest. = total cholesterol, TGs= triglycerides and LDLc = low density lipoprotein cholesterol.
*: Statistically significant at p value ≤ 0.05.
P1= Statistical significance of pre-diabetes when compared with control group
P2= Statistical significance of T2DM when compared with control group.

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P3= Statistical significance of T2DM when compared with pre-diabetes.

Laboratory data in the studied groups

There was a significant statistical increase of fasting, 2 hours post prandial plasma glucose, HbA1c (%), TC and LDL in T2DM group compared to both group patients with pre-diabetes and control groups and in pre-diabetic group when compared to group control group ($p<0.05$). Significant statistical decrease was detected as regards HDL-c in group 3 when compared to both of group 1,2 with non- significant differences in group 2 when compared to group 1 ($p<0.05$). As regard TG, there was a significant statistical increase in group 3 when compared to group 1, 2 with non- significant differences in group 2 when compared to group 1 ($p<0.05$) as demonstrated in, table (2).

Table (3): Comparison of CircRNA 0054633 expression levels between the studied groups

	Group1 Control N=36	Group2 Pre-diabetes N=36	Group3 T2DM N=36	F test	Sig.	P1	P2	P3
CircRNA 0054633expression level	1.1±0.12	2.9±0.35	5.2±0.6	903.8	<0.001*	<0.001*	<0.001*	<0.001*

F test: one way ANOVA. CircRNA = circular ribonucleic acid.
*: Statistically significant at p value ≤ 0.05.
P1= Statistical significance of pre-diabetes when compared with control group.
P2= Statistical significance of T2DM when compared with control group.
P3= Statistical significance of T2DM when compared with pre-diabetes.

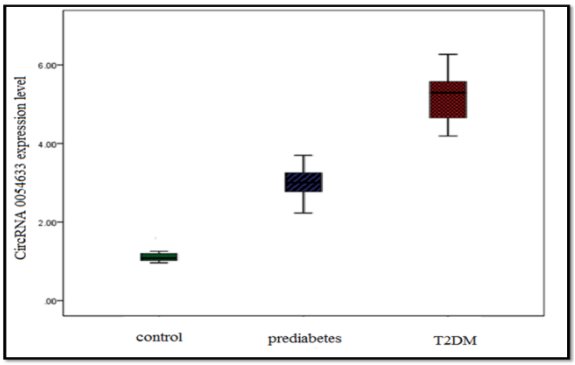

Figure (1): Relative CircRNA 0054633 blood expression levels of in the three studied groups

Expression Levels ofCircRNA 0054633in the studied group

There was a significant up-regulation ofCircRNA0054633 expression levels from group 1 with mean±SD =1.1±0.12 to group 3 with mean±SD=5.2±0.6 with group 2 in-between with mean±SD =2.9±0.35($p<0.05$),table (3) andfigure (1).

Table (4): Linear regression analysis to test the effect of the main independent variables against CircRNA 0054633 expression level (dependent variable)

	Unstandardized Coefficients		Standardized Coefficients	t	P value	95% C.I	
Model	B	SE	Beta			Lower Bound	Upper Bound
Constant	56.93	69.02		0.83	0.42	-84.2	198.1
BMI	0.13	2.70	0.11	0.05	0.96	-5.39	5.66
FBG	-0.38	0.40	-1.71	-0.95	0.35	-1.19	0.44

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HbA1c	0.01	0.004	0.34	2.13	*< 0.05*	0.00	0.02
HDLc	-0.96	1.10	-2.20	-0.87	0.39	-3.21	1.29
LDLc	0.21	0.35	1.53	0.59	0.56	-0.51	0.93
TG	-0.05	0.08	-2.37	-0.62	0.54	-0.22	0.12

BMI= body mass index. FPG= fasting plasma glucose, HbA1c= Glycated hemoglobin, HDL-c = high density lipoprotein cholesterol, TGs= triglycerides and LDLc = low density lipoprotein cholesterol.
*Statistically significant (P < 0.05).

Linear regression analysis with expression levels of CircRNA 0054633 as a dependent variable

Linear regression analysis showed that the increase in HbA1c was independently associatedwith the elevated plasma CircRNA 0054633 expression level,table (4).

Table (5): Correlations of CircRNA 0054633 expression levels with anthropometric and biochemical characteristics in pre-diabetic patients

Characteristics	CircRNA 0054633 expression levelsr	P
-BMI (Kg/m^2)	0.13	0.46
-FPG (mg/dl)	0.08	0.65
-2hPPG (mg/dl)	0.08	0.63
-HbA1c(%)	0.51	0.001
-T.Cholest. (mg/dl)	0.07	0.65
-HDL-c (mg/dl)	-0.01	0.95
-LDLc (mg/dl)	0.085	0.62
-TAGs. (mg/dl)	0.064	0.7

** Correlation is significant at the 0.01 level (2-tailed).

Correlations of CircRNA 0054633 expression levels with anthropometric and biochemical characteristics in pre-diabetic patients

CircRNA 0054633 expression levelswere significantly positively correlated with HbA1c, with non-significant positive correlation with FBG, 2hPPg, total cholesterol, LDL-c, TGs among pre-diabetic patients.There was non-significantly negative correlation between CircRNA 0054633 expression levels and HDL-c among pre-diabetic patients,table (5).

Table (6): Correlations of CircRNA 0054633 expression levels with anthropometric and biochemical characteristics in T2DM patients

Characteristics	CircRNA 0054633 expression level r	P
-BMI (Kg/m^2)	0.089	0.6
-FPG (mg/dl)	0.53	0.001
-2hPPG (mg/dl)	0.09	0.58
-HbA1c(%)	0.5	0.002
-T.Cholest. (mg/dl)	0.09	0.57
-HDL-c (mg/dl)	-0.1	0.53
-LDLc (mg/dl)	0.07	0.67
-TAG. (mg/dl)	0.095	0.58

** Correlation is significant at the 0.01 level (2-tailed).

Correlations of CircRNA 0054633 expression levels with anthropometric and biochemical characteristics in T2DM patients

CircRNA 0054633 expression levelswere significantly positively correlated with FBG and HbA1c with non-significant positive correlation with 2hPPg, total cholesterol, LDLc,TGs

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among T2DM patients.There was non-significantly negative correlation between CircRNA 0054633 expression levels and HDL-c among T2DM patients, table (6).

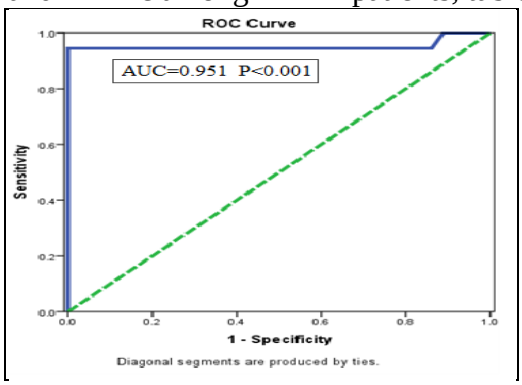

Figure (2): ROC analysis showed that the blood expression level of CircRNA 0054633 discriminating between pre-diabetic patients and controls

Accuracy of circRNA 0054633 expression for discriminatingpre-diabetes from controls by ROCanalyses

The cut-off value of circRNA 0054633 expression determined by ROC analysis to discriminate pre-diabetics from controlswas 1.63, and the AUC was 0.951($p<0.05$).The sensitivity and the specificity of circRNA 0054633expression were 94.4% and 97.2% retrospectively with PPV of 91.8 %, NPV of 94.2% and accuracy of 93% figure (2).

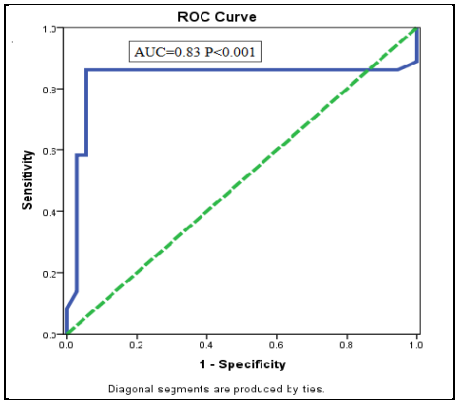

Figure (3): ROC analysis showed that the blood expression level of CircRNA 0054633 when discriminating between T2DM patients and pre-diabetics.

Accuracy of circRNA 0054633 expression for discriminatingT2DM patients from pre-diabetes by ROCanalyses

The cut-off value of circRNA 0054633 expression determined by ROC analysis to discriminate T2DM patientsfrom pre-diabetics was 3.94, and the AUC was 0.83 ($p<0.05$). The sensitivity and the specificity of circRNA 0054633 expressions were 86.1% and 94.4% retrospectively with PPV of 93.9%, NPV of 87.1% and accuracy of 90.3%,figure (3).

Discussion

WHO considered DM as one of the main non-communicable diseases, besides malignancy and the cardiovascular disease [22]. Patients with T2DM often have various complications that severely affect their quality of life and threaten human health [23]. In the coming decades, DM as a rapidly growing global epidemic is predictable to be the chief cause of death as a result of population ageing anddramatic changes in life style [24]. In Egypt, Diabetes is a rapidly progressive health problem with a major effect on morbidity, mortality,

and health care resources. Hegazi et al. reported the prevalence of T2DM in Egypt is about 15.6% of all individualsbetween 20 to 79 years old[4]. According to large scale of investigations, it was revealed that drugs decreasing glucose concentration in the initialphases of T2DM can help patients substantively, decreasing the occurrences of macro-vascular and micro-vascular complications [23].Pre-diabetics have on symptoms, so their need to hospitals to search for diagnosis and treatment isinfrequent. Approximately all individuals with impaired glucose tolerance turn to diabetics later showing the significance of plans for them to permit early diagnosis and hence avoidance or postponement the occurrence of T2DM and its complications [12]. It was found that there is a great association between alterations in the expression of protein-coding and non-coding transcripts and β-cell dysfunction and failure.There is already strong evidence representing the role of different kindsof short non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs in the regulation of β-cell functions and in the progress of DM[25]. In mammalian cells, there were thousands of abundant, endogenous circRNAs with a covalently closed loop transcripts which is formed by direct backsplicing in a non-canonical order [26]. The expression levels of CircRNAs in some tissues are ten times higher than linear RNAs in addition to their more stability in cells, so circRNAs become better biomarkers [27]. RNA-sequencing of human tissues suggested that about 50% of circRNAs are tissue-specific with higher expression levels in fetal tissues than in adult tissues [28]. However, Oral glucose tolerance test (OGTT) is known as the gold standard for detection of pre-diabetes and T2DM, it is time consuming, annoying and complex. Estimation of fasting blood glucose is a suitabletest for diagnosis of T2DM, but the count of lost cases is high [29]. Changes in red blood cell life span as in Hemoglobinpathies and persistent HbF and glycaemia or glycation rates seem to affect HbA1c% [30]. These present methods are insufficiencies for the prediction and early diagnosis of pre-diabetes and T2DM [31].So many researchers have tried to detect convenient, highly specific and sensitive marker for T2DM at early phases [32]. The aim of present study was to study expression profile of circRNA (0054633) in the plasma and to investigate its role in pre-diabetes and T2DM. In our study, fasting, 2hours post prandial blood glucose levels and HbA1c% were significantly higher in both pre-diabetics and T2DM groups compared with the control group.This is in agreement with the results obtained by Cho et al. [33] and Graham et al. [34]. The present study reported that there was a significant statistical increase of total cholesterol and LDL-c in patients with T2DM when compared with controls and pre-diabetics and in pre-diabetics when compared to controls. Significant statistical decrease was detected as regards HDL-c in T2DM when compared to with controls and pre-diabetics with non- significant differences in pre-diabetics when compared to controls. As regard TG, there was a significant statistical increase in T2DM when compared to with controls and pre-diabetics with non- significant differences in pre-diabetics when compared to controls. This comes in line with Songa et al. [35] who found that in patients with T2DM, triglycerides are often elevated, HDL-c is generally decreased, and LDL-c may be elevated, borderline, or normal. As they reported that patients with T2DM having insulin resistance stimulating the expression of hepatic lipase which acts on HDL-c, producing smaller HDL particles that are more rapidly catabolized by the kidney leading to decrease in plasma HDL-c. Also, these results come with agreement with Ozder [36] who suggested that there were extensive lipid defects in the course of diabetes. Multiple studies showed the major role of insulin in formation of liver apolipoprotein and regulation of the enzymatic activity of lipoprotein lipase and cholesterol ester transport protein that causes dyslipidemia in DM[36].Haffner et al.[37] suggested the relationship between carbohydrates and lipid metabolism as any defect in carbohydrate metabolism leads to lipid metabolism disorder and vice versa.CircRNAstudy has become more orderly and consistent with the rapid development of molecular biological information technology and in-depth

RNA sequencing technology. The three most commonly used research methods in this area are molecular biological methods, genomic methods, and circRNA-specific databases [38]. The present researchshowed significant differences between the bloodcircRNA (0054633) expression levels of type 2 diabetics and that of pre-diabetics and controls. The level of circRNA (0054633) expression increased gradually from the control group with mean and standard deviation (1.1±0.12) to the pre-diabetics with mean and standard deviation (2.9±0.35) to the T2DM group with mean and standard deviation (5.2±0.6). These results were in agreement with Zhao et al.(12) who suggested that; circRNA 0054633 had the highest diagnostic value for pre-diabetes and T2DM among other five obtainable markers revealing highest AUC and lowest P values than other circular RNAs. By performing microarray analysis for blood circRNA expression profile, they found that there were marked variances in the expression profiles of 489 circRNAs between the diabetic and control groups. There were 78 circRNAs up-regulated and 411 were down-regulated in the diabetics [12]. Zhao et al.[12] which is the first study investigating the expression profiles of circRNAs in the peripheral blood of patients with T2DM, considered circRNA 0054633 as possibly highly beneficialmethod for the diagnosis of pre-diabetes and prediction of T2DM. Gene ontology (GO) analysis suggested the role of CircRNA 0054633 in biological processes, such as cell cycle and mitotic cell cycle arrest, and also in molecular catabolism [39]. The cellular life activities are regulated by cell cycle process, so regulation of β-cells proliferation is done by cell cycle progress. T2DM which is characterized by insufficient insulin secretion is occurred as a result of decreased β-cell proliferation [40]. El-Hefnway et al.[41] supporting our study that suggested significant differences between the plasma circRNA 0054633 expression levels of T2DM and that of pre-diabetics and controls. The level of circRNA 0054633 expression augmented gradually from the control group to the pre-diabetics to the T2DM group, with a fold change of 1.8 between the first two groups and 2.1 between the latter two groups. Wu et al. [42] reported the relation between gestational diabetes (GDM) and circRNA 0054633 by detecting circRNA 0054633 expression level elevation in the serum of pregnant women in the third trimester of pregnancy and its positive correlation with postprandial blood glucose and glycosylated haemoglobin. This circRNA participates in the regulation of maternal serum glucose metabolism affecting the epigenetic expression of placenta.Brett et al. [43] suggestedthe role of placental epigenetic changes which affect the maternal diet and metabolism of GDM. Pan et al. [44] suggested the role of circ-0054633 expression in suppressing oxidative stress and inflammation by target miRNA-218 and inhibits its expression. It has been revealed that miRNA-218 induces HO-1 down-regulation which act as a protector against high glucose induced cell toxicity leading to their apoptosis [45]. Our study detected no association between expression level of circRNA 0054633 and lipid profile parameters. These results became with agreement with Wu et al. [42] who found no relationship between lipid metabolism and the tested circRNAs among pregnant women.Bai et al. [46] detected new areas for the Autophagy of islet β-cells in T2DM, thus givingnovel ideas for the management of T2DM.Xu et al. [47] found that some circRNA can regulate transcription of insulin and islet cells secretion of transgenic mouse by targeting over expressed microRNA that cause diabetes improving insulin secretion and could become a noveltarget for improving β cell function in diabetes. Xia et al. [48] suggested the participation of circRNA in the Mitogen-activated protein kinase (MAPK) pathway which has a crucial role in gene expression regulation, cytoplasmic activities and Autophagy regulation affecting the occurrence and progression of diabetes.

Conclusion

Plasma circRNA 0054633 expression could be considered as a predictive biomarker for pre-diabetes and hence it is a valuable convenient method for early recognition of T2DM. We recommend further researches to

evaluate circRNA as new target to decrease the development of pre-diabetes into diabetes and to postponement the occurrence of diabetes mellitus complication.

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