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Cell-free long non-coding RNAs (LY86-AS1 & HCG27_201and GAS5) as biomarkers for pre-diabetes and type 2 DM in Egypt

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ABSTRACT

Background: Increasing interest has been focused on lncRNAs as potential markers in the pathogenesis and progression of numerous diseases.
Aim: We aimed to investigate the expression pattern and role of cell-free lncRNAs (GAS5, HCG27_201 and LY86-AS1) in pre-diabetic, diabetic and T2DM groups.
Subjects & methods: Quantification of the expression level of cell-free lncRNAs (GAS5, HCG27_201 and LY86-AS1) was performed by real-time PCR in 210 individuals classified in diabetic (T2DM), pre-diabetic and control groups.
Results: Significant differences were observed in the relative expression level of lncRNAs (GAS5, LY86-AS1 and HCG27_201) among the three studied groups. The LncRNA expression levels decreased gradually from the control to the pre-diabetic group and reached the lowest values in the T2DM group. The A receiver operating characteristic curve (ROC) was applied to identify a cut-off value for each of the three genes among our groups. The three lncRNAs showed promising results in discriminating between the diabetic patients and controls, with HCG27_201 gene expression having the best performance. Furthermore, lncRNA expression was able to predict the future development of DM in the pre-diabetics because ROC analysis among diabetics and pre-diabetics revealed considerable results. GAS5 gene expression showed the best performance. Additionally, HCG27_201 expression was the most valuable biomarker for differentiating between pre-diabetics and controls and presented a sensitivity of 91% and specificity of 64%.
Conclusions: We concluded that cell free lncRNAs (GAS5, LY86-AS1 and HCG27_201) could be considered promising diagnostic and predictive biomarkers for DM and that HCG27_201 could act as a potential diagnostic biomarker for pre-diabetes.

1. Introduction

Diabetes mellitus (DM) is a chief health burden that includes the progression of metabolic disorders recognized by increased levels of blood glucose. Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes and accounts for approximately 90% of all diagnosed diabetics [1]. Initially, the primary phases of T2DM was known as pre-diabetes, in which pancreatic β-cells produce excess insulin in response to hyperglycemia. Finally, the β-cell depletes itself, insulin generation diminishes and T2DM is manifested [2]. T2DM risk is multifactorial and caused by the interaction of genetic, environmental, and developmental elements [3]. Moreover, the impact of T2DM is complicated by its late diagnosis after development of disease complications [4]. Recent studies revealed that only 20,000 genes among whole genomic sequences code for proteins, and this value represents only approximately 2% of the human genome [5]. The products of the noncoding genes (98% of the genome transcript) are called noncoding RNAs (ncRNAs), which are further classified into transcription RNAs (tRNA and rRNA), short noncoding RNAs (miRNA, snRNA) and long noncoding RNAs (lncRNAs). lncRNAs have lengths more than > 200 nucleotides and show DNA-, RNA- and protein-binding abilities [6]. Although the significance of most of lncRNAs has not been identified, various studies have reported that they function in the regulation of gene expression, cellular differentiation and various diseases [7]. Additionally, lncRNAs have vital roles in cellular signaling, scaffolding, ribonuclear decoys and cell cycle progression [8]. Various lncRNAs have been shown to be involved in the T2DM pathological process. An investigation of the transcript of human β-cells in T2DM revealed the aberrant expression of

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lncRNAs [3,9]. Growth arrest-specific 5 (GAS5) is a lncRNA, and its gene is located at 1q25, a location that is associated with abnormalities in many malignancies, such as melanoma and prostate cancer as well as in systemic lupus erythematosus [10,11]. GAS5 expression is correlated with DM and emerging as a new biomarker of DM risk [12]. Additionally, GAS5 functions as ribo-repressor because it represses glucocorticoid receptor transcription [13]. The GAS5 transcript concentrations are controlled by RNA degradation through the non-sense mediated decay (NMD) mechanism and not by modulation during transcription [14]. Additionally, the expression levels of the lncRNAs LY86-AS1 and HCG27_201 were suggested to be involved in the development of T2DM and LY86-AS1 and thus might be used as potential diagnostic biomarkers for T2DM [15]. We aimed to study the expression pattern and detect the role of lncRNAs (GAS5, LY86-AS1 and HCG27_201) in type 2 DM and pre-diabetic patients in Egypt.

2. Subjects and methods

2.1. Study subjects

This study was performed via co-operation between the Medical Biochemistry & Molecular Biology and Endocrinology units of the Internal Medicine Department, Menoufia Faculty of Medicine, from June 2017 to November 2019. The study cohort included 210 individuals classified as follows: group I: 70 patients with T2DM; group II: 65 pre-diabetics individuals; and group III: 70 healthy controls. Diabetics were diagnosed according to criteria of the American Diabetes Association (ADA). The following criteria were required for a diagnosis with T2DM: (i) fasting plasma glucose (FPG) level of 126 mg/dl or greater (fasting is identified as no caloric input for at least 8 h); (ii) 2-h post prandial glucose level (2-HPPG) of 200 mg/dl or greater during a 75-g OGTT or a random plasma glucose level of 200 mg/dl or greater in a patient with characteristic symptoms of hyperglycemia (e.g., polyuria, polydipsia, polyphagia, weight loss); and (iii) a hemoglobin A1c (HbA1c) level of 6.5% or more. Furthermore, patients with any of the following criteria were diagnosed as pre-diabetes: (i) FPG of 100 mg/dl to 125 mg/dl [impaired fasting glucose (IFG)]; and (ii) 2-HPPG in the 75-g OGTT of 140 mg/dl to 199 mg/dl [impaired glucose tolerance (IGT)] and a hemoglobin A1c (HbA1c) level of 5.7–6.4% [16]. Complete medical records were obtained for all study group participants, who were subjected to a full clinical examination, including anthropometric measurements. The body mass index (BMI) was calculated by dividing the body weight in kilograms by the height in square meters [17]. Laboratory investigations included determining the fasting and 2 h post prandial blood glucose levels, glycated hemoglobin (HbA1c %), lipid profile [serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) calculations], and fasting serum insulin levels and assessing insulin resistance by the homeostatic model assessment of insulin resistance (HOMA-IR). Quantification of the cell-free long noncoding RNA (LY86-AS1 & HCG27_201 and GAS5) expression levels was performed by the real-time PCR technique.

2.2. Ethical approval

Blood samples were taken from participants enrolled in this study after obtaining written consent and approval from the university research ethics committee of Menoufia Faculty of Medicine.

2.3. Blood sampling

After 12 h of overnight fasting, 10 ml of venous blood was withdrawn from every subject and distributed into three samples. The first sample included 4 ml of blood subdivided into 2 EDTA tubes, with one used for the quantitative colorimetric assessment of HbA1c% utilizing kits from Teco Diagnostics (USA) and the other centrifuged in a cooling centrifuge at 4 °C for 15 min at 4000 r.p.m. The separated fresh plasma was used for RNA extraction. The second sample consisted of 1 ml of blood that was transferred into a sodium fluoride tube with another sample of blood that was taken after 2 h to measure blood glucose by the enzymatic colorimetric method using a via Spinreact kit (SPAIN). The third sample consisted of 5 ml of blood that was transferred into a plain tube, subjected to serum separation and then kept frozen at −80 °C until the determination of serum TC, HDL-c and TG. Serum TC and TG were assessed by the enzymatic colorimetric test using a Spinreact kit (SPAIN). Serum HDL-c was assessed by the colorimetric method using a Human kit (GERMANY). LDL-c was estimated from the TC concentration, HDL-c and TG according to Ref. [18]. Serum insulin was determined by the enzyme-linked immunosorbent assay method using a DRGR Insulin ELISA kit (GERMANY) [19]. Insulin resistance was assessed by the homeostatic model assessment (HOMA) according to Ref. [20]. HOMA - IR = fasting glucose (mg/dl) × fasting insulin (μIU/mL)/405.

2.4. RNA isolation from fresh plasma & reverse transcription

Total RNA in plasma was isolated using a GeneJET RNA Purification Mini Kit (Thermo Scientific, USA). The concentrations of RNA were determined by a NanoDrop™ 2000 (Thermo Scientific, USA). RNA extract was stored at −80 °C until the reverse transcription step. A RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) was used for the reverse transcription step and production of complementary DNA (cDNA). Reactions were performed on ice with a net volume of 20 μl in two-step reactions as follows: first, 10 μl of template RNA was added to 1 μl of random hexamer and 1 μl nuclease free water to attain a total volume of 12 μl, which was then incubated at 65 °C for 5 min and then put in ice; second, 4 μl of 5 × reaction buffer, 1 μl ribolock Rnase inhibitor, 2 μl of 10 MM dNTP Mix and 1 μl of revertaid RT was added to the aforementioned mix to attain a total volume of 20 μl. Incubation was carried out on a 2720 thermal cycler (Applied Biosystems, Singapore) in single cycles as follows: 25 °C for 5 min, 42 °C for 60 min, and termination at 70 °C for 5 min. The cDNA was kept at −20 °C until the real-time PCR step.

2.5. Quantification of LncRNA expression by real time PCR technique

Real-time PCR was performed using a SensiFASTTM SYBR Lo-ROX Kit, USA and a total volume of 20 μl, which included 10 μl of SYBR green Master Mix; 1 μl of nuclease-free water, 6 μl of template cDNA and 1.5 μl of each forward and reverse primer. The primer sequence was confirmed with the National Centre for Biotechnology Information (NCBI). Accordingly, the following primers (Midland, Texas) were used. For the LY86-AS1 gene: forward 5′-TGGAGAGCAAGAACTATAGGAGGA-3′ and reverse 5′-TTGACCAGACTACAGACATAGCAC-3'; for the HCG27_201 gene: forward 5′-CCAGGAAAGTGAAAAGAGAAGCAG-3' and reverse 5′-GTTTCATCCTACCACTCCCAATTAAT-3'; for the GAS 5 gene: forward 5′-AGCTGGAAGTTGAAATGG-3', and reverse 5′-CAAGCCGACTCTCCATACC-3'; and for the housekeeping gene (GAPDH): forward 5′-GAAGGTGAAGGTCGGAGTC-3′, and reverse 5′-GAAGATGGTGATGGGATTTC-3'. The gene amplification conditions consisted of 3 phases: preliminary phase at 95 °C for 5 min; then 50 cycles at 95 °C for 15 s, 60 °C for 1 min and 72 °C for 1 min; and a final extension phase at 72 °C for 10 min. Finally, fluorescence detection and data analysis were completed using a 7500 ABI PRISM system (Applied Biosystems, USA) v.2.0.1. The $2^{-\Delta\Delta Ct}$ method was utilized to compute the relative expression of the lncRNAs as normalized to the endogenous housekeeping gene (GAPDH) and relative to the control, with ΔCt = Ct target – Ct reference, −ΔΔCt = − (sample ΔCt – control ΔCt) [21].

2.6. Statistical analysis

The data were analyzed on an IBM PC using SPSS version 22 (SPSS

Inc., Chicago, Illinois, USA). An ANOVA (f) was performed to compare three or more groups with quantitative variables. The Kruskal-Wallis test was used to compare three groups with non-normally distributed quantitative variables. Spearman's correlation (r) is a test used to measure the association between two quantitative nonparametric variables. A receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of our parameters, such as the sensitivity and specificity at different cut-off points. A p-value < 0.05 was considered significant.

3. Results

The current analysis investigated 210 individuals categorized into 3 groups. Group I enrolled 70 patients diagnosed with T2DM, with a mean disease duration (2.46 ± 2.28) years, and 68 (90.7%) of these patients of had a positive family history for DM; Group II enrolled 65 pre-diabetic patients; and Group III enrolled 70 healthy controls were enrolled as the control group. The analysis of the data from our investigation showed no significant differences in age ($p = 0.456$) and sex ($p = 0.974$) among the three groups, thus revealing consistency in age and gender. However, the ANOVA test and post hoc test reported that significant variations ($p = 0.001$) occurred among the three groups and in the diabetic patients compared to the controls in terms of BMI, FBG, 2 h-PG, HbA_{1c}, HDL-c, TC, TGs and LDL-c. Additionally, the diabetic patients showed significant ($p = 0.001$) increases in BMI, FBG, 2HPPG, HbA_{1c}, TG, fasting insulin and HOMA-IR compared with the pre-diabetic group. A comparison of the pre-diabetic group to the controls revealed that a significant ($p = 0.001$) increase in FBG, 2 h-PG, TC, LDL-c, fasting insulin, HOMA-IR and decrease in HDL-c levels occurred in the pre-diabetics, while no significant variations occurred in the BMI, HbA1c and TG values ($p = 0.347$, $p = 0.126$ and $p = 0.110$ respectively) (Table 1).

A comparison of the relative expression levels of the lncRNAs (GAS5, LY86-AS1 and HCG27_201) among our groups revealed significant variations in their expression levels ($p = 0.001$). The diabetic patients reported the lowest expression level of GAS5, LY86-AS1 and HCG27_201 compared to the pre-diabetics and controls ($p = 0.001$). Furthermore, the pre-diabetic group reported reduced expression levels of GAS5 ($p = 0.001$), LY86-AS1 ($p = 0.002$) and HCG27_201 ($p = 0.001$) when compared to the control group (Fig. 1).

Table 2 indicates that significant correlations in the expression levels of the selected lncRNAs occurred with various clinical parameters. LncRNA GAS5 expression showed a positive correlation with age ($r = 0.269$, $p = 0.030$) in the pre-diabetic group and negative correlation with FBG ($r = -0.313$, $p = 0.007$) and 2-HPPG ($r = -0.252$, $p = 0.029$) in diabetic patients. Regarding LY86-AS1gene expression, data analysis detected negative correlation with HbA_{1c}% in both diabetic ($r = -0.353$, $p = 0.002$) and pre-diabetic ($r = -0.252$, $p = 0.043$) groups and with TC ($r = -0.281$, $p = 0.023$) in the pre-diabetic-diabetics. The relationships of lncRNA HCG27_201 expression with the studied parameters showed negative correlations with both FBG ($r = -0.343$, $p = 0.003$) and 2-HPPG ($r = -0.281$, $p = 0.015$) in diabetic patients and had negative correlation with BMI ($r = -0.288$, $p = 0.020$) in the prediabetic-diabetics.

Table 3 shows that significant positive correlations occurred between the fasting insulin and FBG, 2-HPPG, HbA1c%, total cholesterol and LDL-c in the diabetic group and between the fasting insulin and TG in the pre-diabetic group while a significant negative correlation was detected between the fasting insulin and LDL-c in the pre-diabetic groups. HOMA-IR showed significant positive correlations with FBG, 2-HPPG, HbA1c%, total Cholesterol and LDL-c in the diabetic group and with TG in the pre-diabetic group. A significant negative correlation was detected between HOMA-IR and HDL-c in the diabetic group, while a significant negative correlation was detected between HOMA-IR and LDL-c in the pre-diabetic group.

To examine whether the expression of the three cell-free lncRNAs expression might be useful as potential biomarkers for T2DM and pre-diabetes diagnoses, the ROC curve was applied to identify a cut-off value for each of the three genes among our groups. The three lncRNAs showed promising results in discriminating between the diabetic patients and controls. The expression of the HCG27_201 gene had the best performance with a cutoff value ≤ 0.75, (AUC = 0.957, p= < 0.001), sensitivity of 96% and specificity of 83%. The GAS5 gene expression cutoff value was ≤0.76, (AUC = 0.938, p= < 0.001), with sensitivity of 96% and specificity of 76%. For LY86-AS1 gene expression, the best cutoff value was ≤0.67, (AUC = 0.831, p= < 0.001), with sensitivity of 93% and specificity of 69% (Table 4 and Fig. 2). Furthermore, LncRNA expression showed the ability to predict the future development of DM in the pre-diabetics based on the ROC analysis among diabetics and pre-diabetics. GAS5 gene expression showed the best performance, with a cutoff value ≤ 0.71 (AUC = 0.966, p= < 0.001), sensitivity of 96% and specificity of 78%. The LY86-AS1 gene expression cutoff value was ≤0.66, (AUC = 0.946, p= < 0.001), with sensitivity of 93% and specificity of 88%. While for HCG27_201 gene expression, the best cutoff value was ≤0.68 (AUC = 0.840, p= < 0.001), with sensitivity of 91% and specificity of 83% (Table 5 and Fig. 3). To differentiate pre-diabetic patients from healthy controls, HCG27_201 expression was the most valuable biomarker, with a cutoff value ≤ 0.98, (AUC = 756, p= < 0.001), sensitivity of 91% and specificity of 64%, whereas the lncRNAs GAS5 and LY86-AS1 were less valuable because they reported a cutoff value ≤ 0.99 (AUC = 0.699, p= < 0.001), sensitivity of 85% and specificity of 64% and a cut off value ≤ 0.93 (AUC = 0.602, p= < 0.001), sensitivity of 78% and specificity of 53%, respectively (Table 6 and Fig. 4).

4. Discussion

OGTT is a high-value test for the diagnosis of pre-diabetes and T2DM. However, this test is time consuming, inconvenient and complicated. Fasting plasma glucose is a convenient tool for T2DM diagnosis, although the frequency of missed diagnosis is high [22]. Current methods have several inadequacies for early detection and prediction of pre-diabetes and T2DM [23]. Pre-diabetics are asymptomatic and rarely visit clinics for diagnosis and treatment [24]. Nearly all pre-diabetics become diabetics after a variable period, thus highlighting the importance of developing strategies for these individuals to allow for early detection and the prevention or delay in the development of T2DM and its complications [25]. Therefore, numerous studies have tried to identify suitable, highly specific and sensitive biomarkers for T2DM at early stages [26]. Despite the recent widely investigated relationship of lncRNAs and progression of various diseases, the irregular expression and role of lncRNAs in glucose homeostasis and T2DM are still basically unidentified [27]. Therefore, we aimed to assess the expression levels of lncRNAs (GAS5, LY86-AS1 and HCG27_201) in T2DM and pre-diabetic patients in Egypt.

Our study revealed significant differences in the relative expression level of lncRNAs (GAS5, LY86-AS1 and HCG27_201) in type 2 diabetics comparing to that of pre-diabetics and controls. The LncRNA expression level decreased gradually from the control group to the pre-diabetics and reached lowest levels in T2DM group. These results are consistent with **Saeidi et al. (2018)** [15], who conducted a study on T2DM patients and healthy controls and found a significant reduction in the expression of both HCG27-201 and LY86-AS1 in the T2DM group compared with the controls in an Iranian population. Similarly, **Carter**

Table 1
Demographic data and laboratory investigation of the studied groups.

Studied variables	Diabetic group (n = 75)	Pre-diabetic group (n = 65)	Controls (n = 70)	F	p value	Post hoc test
Age/years	53.7 ± 7.34	52.3 ± 6.48	52.5 ± 7.48	F =	0.456	p1:0.250
Mean ± SD	41–68	42–67	40–67	0.787		p2:0.328
Range						p3:0.849
Sex	n (%)	n (%)	n (%)	X2 =	0.974	–
Male	40 (53.3)	34 (52.3)	38 (54.3)	0.053		
Female	35 (46.7)	31 (47.7)	32 (45.7)			
BMI (kg/m^2)	27.4 ± 2.53	22.6 ± 0.70	22.3 ± 2.41	F =	0.001**	p1:0.001**
Mean ± SD	22–32	21–24	18–28	134.8		p2:0.001**
Range						p3:0.347
FBG (mg/dl)	265.0 ± 73.1	118.9 ± 4.02	88.5 ± 9.04	333.0	0.001**	p1:0.001**
Mean ± SD	146–416	112–124	74–103			p2:0.001**
Range						p3:0.001**
2-HPPG (mg/dl)	296.6 ± 77.2	157.1 ± 14.1	88.2 ± 8.46	369.8	0.001**	p1:0.001**
Mean ± SD	178–444	135–195	72–104			p2:0.001**
Range						p3:0.001**
HbA1c (%)	10.4 ± 1.27	5.59 ± 1.08	5.21 ± 0.84	525.3	0.001**	p1:0.001**
Mean ± SD	8.30–12.9	3.00–6.30	3.00–6.80			p2:0.001**
Range						p3:0.126
HDLc (mg/dl)	31.9 ± 1.38	32.3 ± 3.08	48.5 ± 1.21	1130.0	0.001**	p1:0.322
Mean ± SD	29–34	28–39	46–50			p2:0.001**
Range						p3:0.001**
T. cholest. (mg/dl)	208.1 ± 25.5	210.9 ± 15.4	172.1 ± 9.23	96.9	0.001**	p1:0.353
Mean ± SD	172–277	166–226	155–186			p2:0.001**
Range						p3:0.001**
TGs.(mg/dl)	164.7 ± 10.1	95.8 ± 12.1	93.1 ± 4.84	1324	**0.001****	**p1:0.001****
Mean ± SD	143–183	81–116	83–98			**p2:0.001****
Range						p3:0.110
LDL(mg/dl)	143.2 ± 25.2	145.4 ± 15.5	104.9 ± 8.85	110.1	**0.001****	p1:0.455
Mean ± SD	143–183	100–161	88–118			**p2:0.001****
Range						**p 3:0.001****
Fasting insulin (μIU/mL)	21.2 ± 5.24	8.21 ± 5.85	4.00 ± 0.49	K =	**0.001****	**P1:0.001****
Mean ± SD	11–30	3–20	3–5	134.3		**P2:0.001****
Range						**P3:0.001****
Insulin resistance	12.5 ± 7.50	2.20 ± 1.86	0.85 ± 0.11	K =	**0.001****	**P1:0.001****
Mean ± SD	3–30	0.60–6	0.60–1	137.7		**P2:0.001****
Range Mean ± SD						**P3:0.001****
Range						
Disease duration/years	2.46 ± 2.28					
Mean ± SD	0.08–8					
Range						
Family history	**n (%)**					
Positive	68 (90.7)					
Negative	7 (9.30)					

F: ANOVA test; * significant; **highly significant; and K: Kruskal-Wallis test.
p1: Comparison between diabetic and pre-diabetic patients. **p**2: Comparison between diabetic patients and controls. **p** 3: Comparison between pre-diabetic patients and controls.

et al. (2015) [12] reported that circulating lncRNA GAS5 was significantly correlated with diabetes and found reduced GAS5 expression levels in diabetic patients compared to non-diabetic patients. Additionally, they supposed that lncRNAs might act via the repression of insulin resistance as a possible mechanism against hyperglycemia of T2DM.

Sathishkumar et al. (2018) [28] detected a correlation between lncRNAs dysregulation in T2DM and pathological processes manifested by reduced glycemic control, insulin resistance, enhanced cellular senescence, and broad inflammation. Furthermore, **Ge et al. (2019)** [29] stated that lncRNA GAS5 expression was downregulated in diabetic nephropathy and negatively associated with the severity of diabetic nephropathy-related complications. As lncRNA GAS5 inhibits cell proliferation and fibrosis, its silencing contributed to cell proliferation and fibrosis.

Additionally, previous analyses of breast cancer showed that GAS5 levels were altered by mTOR inhibitors [30] and the repression of mTOR affected carbohydrate metabolism [31]. Moreover, lncRNA dysregulation may be involved in T2DM pathological processes by moderating inflammation and insulin resistance [32].

The current analysis also revealed that there was a significant negative correlation between the GAS5 and HCG27_201 expression levels and FBG and 2-HPPG in the diabetic group. These results are consistent with those of **Saeidi et al. (2018)** [15]**,** who also detected a significant negative relationship between LY86-AS1 and HCG27-201 expression and FBG. They reported that this inverse correlation between lncRNA expression and FBG indicated that these two lncRNAs might play a significant a role in the regulation of glucose levels. Compared with the Iranian study by **Saeidi et al. (2018)** [15], who found no relation between the expression of these two lncRNAs and BMI & HbA1c%, we found a significant negative correlation between the LY86-AS1 gene expression level and HbA1c% in both the diabetic and pre-diabetic groups. Additionally, we detected a significant negative correlation between HCG27_201 expression and BMI in the prediabetic-diabetics. These differences could be the effect of ethnicity, discrepancies in the characteristics of study population, study design and degree of glycemic

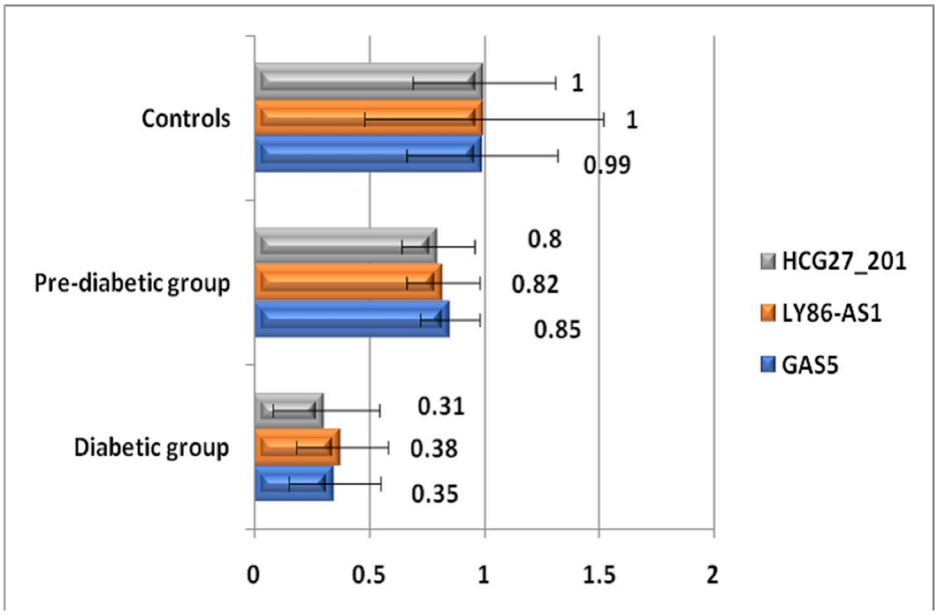

Fig. (1). LncRNA gene expression among the studied groups.

control.

We evaluated which of the three tested LncRNAs could be a marker for discriminating between diabetic patients and controls. HCG27_201 gene expression showed the best performance, with an AUC of 0.957 (sensitivity = 96%, specificity = 83%), while that of GAS5 was 0.938 (sensitivity = 96%, specificity = 76%) and that of LY86-AS1 was 0.831 (sensitivity = 93%, specificity = 69%). The previous study performed by **Saeidi et al. (2018)** [15] revealed that LY86-AS1 had an AUC of 0.747, $p < 0.0001$, sensitivity of 64.6, and specificity of 79.8. Thus, the lncRNA LY86-AS1 could be considered as a reasonable new potential biomarker for DM diagnosis. **Carter et al. (2015)** [12] assessed the circulating GAS5 levels in diabetic patients and non-diabetic controls, and the ROC curve revealed an AUC of 0.08 for GAS5, with sensitivity of 85.1% and specificity of 67.3%.

Additionally, the three selected lncRNAs could effectively predict the future progression to T2DM in the prediabetic-diabetics. The GAS5 gene expression among the three studied lncRNAs had the best performance, with an AUC of 0.966 (sensitivity = 96%, specificity = 78%), while the AUC of LY86-AS1 was 0.946 (sensitivity = 93%, specificity = 88%) and that of HCG27_201 was 0.840 (sensitivity = 91%, specificity = 83%).

Our study was the first to examine the role of lncRNAs in the differentiation of pre-diabetics from healthy controls, and we found that HCG27_201 expression was the most valuable biomarker, with a sensitivity of 91% and specificity of 64%.

5. Conclusions

Three selected lncRNAs (GAS5, LY86-AS1 and HCG27_201) could be considered as promising diagnostic biomarkers for DM and predictive biomarkers for the development of DM in the pre-diabetics. Cell-free HCG27_201 might serve as a potential diagnostic marker for pre-diabetes in Egyptian patients. We recommend that further studies should evaluate lncRNAs as therapeutic targets to decrease the progression of pre-diabetes into diabetes and delay the incidence of diabetic complications.

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CRediT authorship contribution statement

Amany A. Saleh: Writing - review & editing. **Heba E. Kasem:** Writing - original draft. **Enas S. Zahran:** Investigation. **Sally M. El-Hefnawy:** Supervision, Methodology.

Table 2
Correlation between the lncRNA gene expression and the clinical and laboratory data of the studied patients.

Parameters	GAS5 gene				LY86-AS1 gene				HCG27_201 gene			
	Diabetic group		Pre-diabetic group		Diabetic group		Pre-diabetic group		Diabetic group		Pre-diabetic group	
	r	P	R	p	r	p	r	p	r	P	r	p
Age/years	0.087	0.460	**0.269**	**0.030***	−0.035	0.766	−0.003	0.980	−0.205	0.078	−0.221	0.077
Disease duration	0.066	0.574	–	–	−0.135	0.248	–	–	−0.172	0.141	—	——
BMI (kg/m^2)	0.212	0.068	−0.181	0.148	0.145	0.216	0.136	0.279	−0.125	0.284	**−0.288**	**0.020***
FBG (mg/dl)	**−0.313**	**0.006****	−0.192	0.125	−0.043	0.716	0.214	0.087	**−0.343**	**0.003****	0.140	0.265
2-HPPG (mg/dl)	0.128	0.274	0.062	0.624	−0.034	0.773	0.00	0.999	**−0.281**	**0.015***	0.116	0.358
HbA1c (%)	**−0.252**	**0.029***	0.027	0.829	**−0.353**	**0.002****	**−0.252**	**0.043***	−0.054	0.645	−0.094	0.456
HDLc (mg/dl)	−0.122	0.295	0.146	0.245	−0.072	0.540	0.020	0.876	0.075	0.520	−0.132	0.293
T. cholesterol (mg/dl)	−0.021	0.855	0.090	0.474	0.120	0.305	**−0.281**	**0.023***	−0.001	0.996	0.111	0.381
TGs (mg/dl)	0.192	0.100	−0.066	0.601	−0.069	0.555	0.154	0.221	0.027	0.817	−0.211	0.091
LDL (mg/dl)	−0.031	0.794	0.125	0.322	0.152	0.192	0.219	0.080	−0.007	0.956	0.190	0.129

r_s: Spearman coefficient **Highly significant.

Table 3
Correlations among fasting insulin, insulin resistance, lncRNA gene expression and clinical and laboratory data in the studied patients.

Parameters	Fasting insulin (μIU/mL)				Insulin resistance			
	Diabetic group		Pre-diabetic group		Diabetic group		Pre-diabetic group	
	r	p	R	p	r	p	r	p
Age/years	0.057	0.629	−0.052	0.683	0.008	0.945	−0.065	0.604
Diseases duration	0.102	0.385	–	–	0.053	0.652	–	–
BMI (kg/m2)	0.050	0.673	0.077	0.542	0.076	0.516	0.066	0.602
FBG (mg/dl)	0.387	**0.001****	−0.104	0.408	0.517	**0.001****	−0.096	0.448
2-HPPG (mg/dl)	0.369	**0.001****	0.060	0.633	0.475	**0.001****	0.060	0.635
HbA1c (%)	0.387	**0.001****	0.299	0.016*	0.458	**0.001****	0.297	0.016*
HDL-c (mg/dl)	−0.226	0.051	0.131	0.297	−0.333	**0.004****	0.138	0.272
T. cholesterol (mg/dl)	0.356	**0.002****	0.181	0.148	0.346	**0.002****	0.187	0.136
TGs. (mg/dl)	−0.052	0.722	0.757	**0.001****	0.013	0.912	0.763	**0.001****
LDL (mg/dl)	0.376	**0.001****	−0.317	**0.010****	0.367	**0.001****	−0.315	**0.011***
GAS5 gene	−0.185	0.112	−0.071	0.573	−0.155	0.185	−0.083	0.509
LY86-AS1 gene	−0.151	0.196	−0.050	0.691	−0.222	0.055	−0.036	0.774
HCG27_201 gene	−0.125	0.284	0.020	0.874	−0.171	0.141	0.018	0.888

r_s: Spearman coefficient **Highly significant.

Table 4
Validity of lncRNA gene expression for differentiating between diabetic patients and controls.

	AUC	Cut off point	p value	CI 95%	Sensitivity	Specificity	PPV	NPV	Accuracy
GAS5 gene expression	0.938	≤0.76	< 0.001	0.899–0.977	96%	76%	81%	95%	0.86%
LY86-AS1 gene expression	0.831	≤0.67	< 0.001	0.762–0.900	93%	69%	76%	91%	0.81%
HCG27_201 gene expression	0.957	≤0.75	< 0.001	0.926–0.987	96%	83%	86%	95%	0.90%

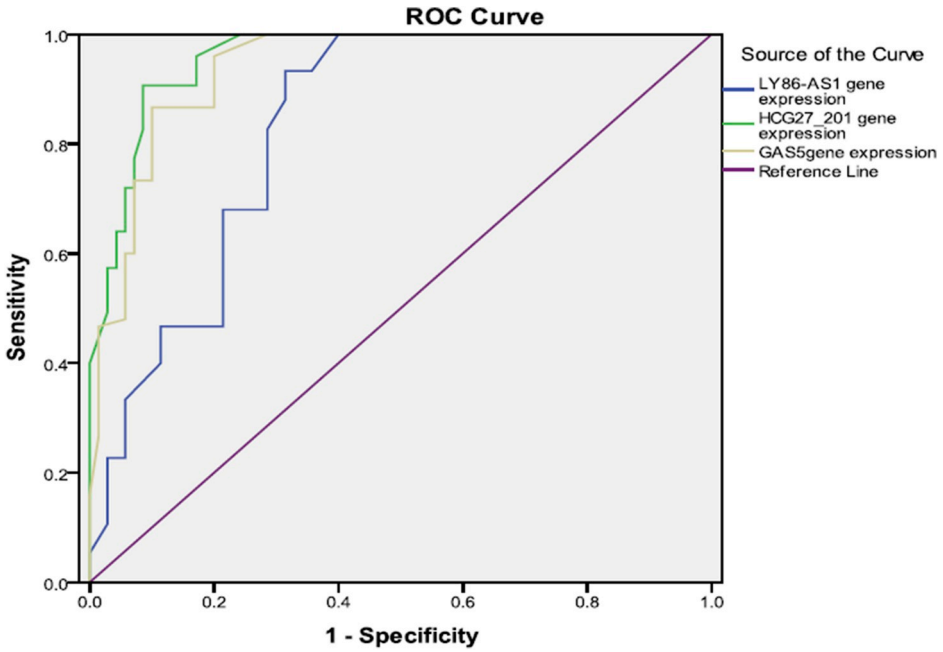

Fig. (2). ROC curves of the expression of the three lncRNAs)GAS5, LY86-AS1 and HCG27_201) to differentiate diabetic patients from controls.

Table 5
Validity of lncRNA gene expression for differentiating between diabetics and pre-diabetic patients.

	AUC	Cut off point	p value	CI 95%	Sensitivity	Specificity	PPV	NPV	Accuracy
GAS5 gene expression	0.966	≤0.71	< 0.001	0.941–0.991	96%	78%	84%	94%	0.88%
LY86-AS1 gene expression	0.946	≤0.66	< 0.001	0.911–0.981	93%	88%	90%	92%	0.91%
HCG27_201 gene expression	0.940	≤0.68	< 0.001	0.906–0.975	91%	83%	86%	89%	0.87%

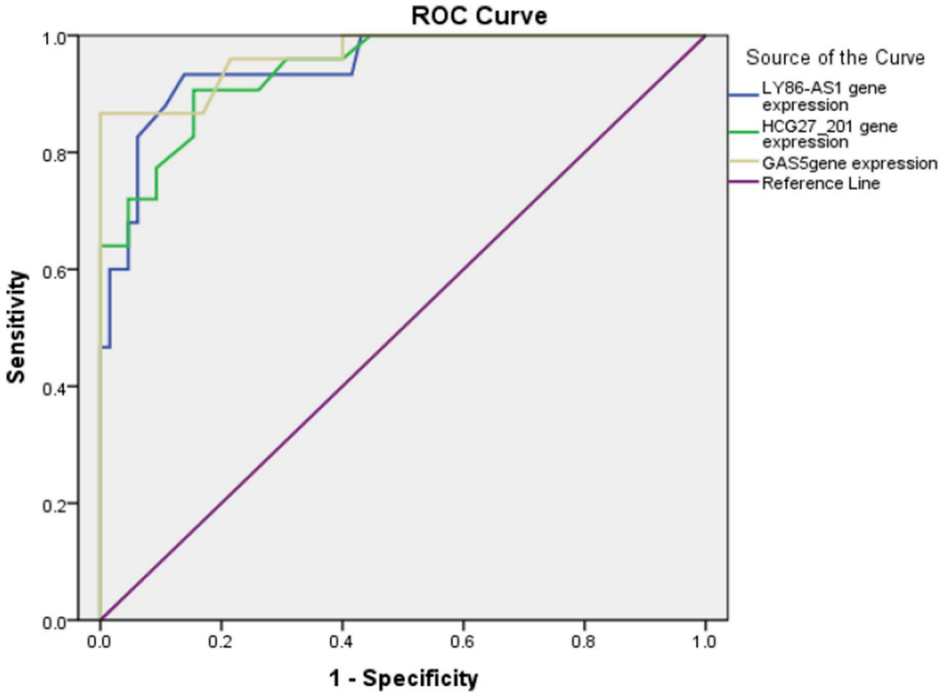

Fig. (3). ROC curves of the expression of the three lncRNAs)GAS5, LY86-AS1 and HCG27_201) to differentiate diabetic patients from pre-diabetics.

Table 6
Validity of lncRNA gene expression for differentiating between pre-diabetics and controls.

	AUC	Cut off point	p value	CI 95%	Sensitivity	Specificity	PPV	NPV	Accuracy
GAS5 gene expression	0.699	≤0.99	< 0.001	0.605–0.792	85%	64%	69%	82%	0.74%
LY86-AS1 gene expression	0.602	≤0.93	0.041	0.497–0.707	78%	53%	61%	73%	0.65%
HCG27_201 gene expression	0.756	≤0.98	< 0.001	0.672–0.841	91%	64%	79%	88%	0.77%

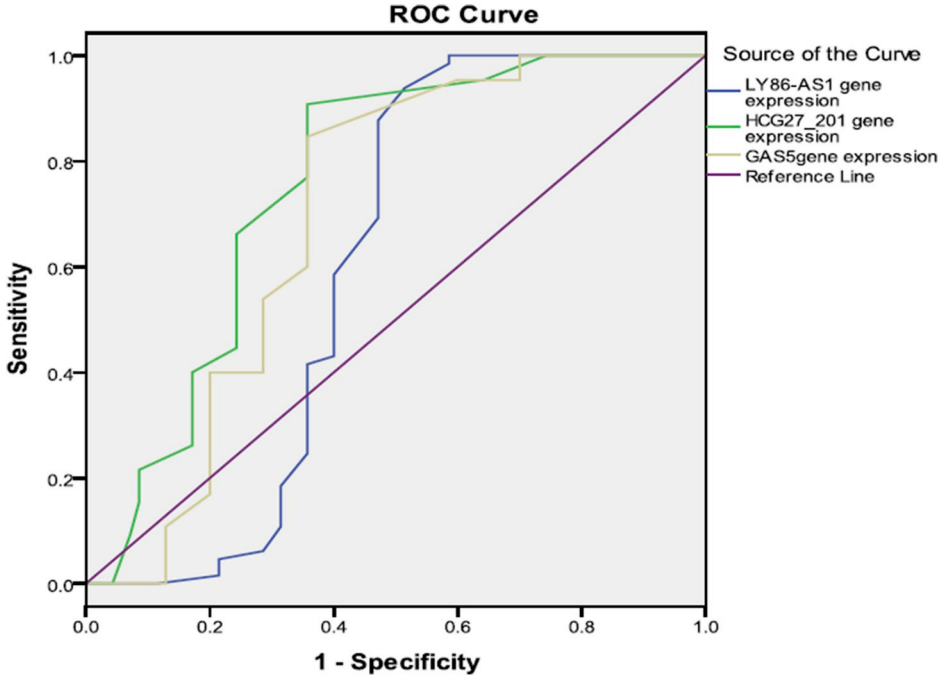

Fig. (4). ROC curves of the expression of the three lncRNAs)GAS5, LY86-AS1 and HCG27_201) to differentiate pre-diabetic patients from controls.

Declaration of competing interest

There is no conflict of interest among authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2020.100770.

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