Dysregulated Circulating micro RNAs Markers: New Evidence into Expression Pattern in Children with T1D among Egyptian Population

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Abstract

Background

miRNAs are gaining access as promising markers for a variety of autoimmune disorders yet, deviations between individuals at risk or developed T1D remains to be thoroughly explored. We aimed to study the pattern of miRNA expression profiling in plasma obtained from patients with T1D and the matched control subjects.

Methods

Equally divided numbers of T1D patients (90) and healthy-matched control children (90) were analyzed for their expression profile of plasma miRNAs; miR-101-5p, 146-5p, 21-5p, miR-375, miR-126, and Let7a-5p by reverse transcriptase (RT-PCR) through quantitative real-time technique.

Results

The two studied groups were significantly different in respect to their biochemical parameters; FBG, 2hpp, and HbA1c levels ($p < 0.05$). Among the deregulated molecules, miR-101, miR-21 and-375 were highly expressed, whereas, miR-146-5p, miR-126, and miR-Let7a-5p showed significantly low levels of expression in patients compared to control subjects ($p < 0.05$). MiR-101, miR-146 was significantly correlated to the age at diagnosis of T1D and disease duration respectively. Furthermore, miR-126, -Let7a-5p showed a significant negative correlation with meanA1C values, the matter that was confined by multivariate analysis.

Conclusion

Dysregulation of the above mentioned micro RNAs pointed out to their pivotal role to be important biomarkers for T1D development.

Background

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder characterized by destruction of pancreatic beta-cells by T-lymphocytes and macrophages [1]. The disease is usually diagnosed when over 80–90% of beta-cells have been destructed by the infiltrating immune system. T1DM development is a slow process providing a potentially long window of time in which it is possible to identify and theoretically treat individuals at risk [2, 3].

It's estimated that about 80,000 children develop the disease each year [4]. Microvascular complications of diabetes have a significant impact on the quality of life, morbidity, and mortality, posing a huge burden on the health care system. Diabetic nephropathy is a leading cause of end-stage renal disease (ESRD) and augments the risk of cardiovascular diseases (CVD). Diabetic retinopathy is the major cause of new blindness in adults. Therefore, it is urgent to identify novel targets for treatment and to discover innovative noninvasive biomarkers to improve risk prediction, early diagnosis, and prognosis assessment [5].

Non-coding RNAs including microRNAs (miRNAs) play an important role in the pathogenesis of T1D. miRNAs are short (~ 22 nucleotides) non-coding RNAs that regulate gene expression in a posttranscriptional manner [6]. In

general, the miRNAs exert their functions via binding with the 3' untranslated regions (UTRs) of their target genes, resulting in translational inhibition or direct degradation of the targeted mRNA leading to diminished protein expression [6, 7]. Alterations in miRNA expression have been associated with several human autoimmune and inflammatory diseases including T1D [8, 9].

MiRNAs regulate the expression of more than 60% of protein-coding genes; consequently, changes in their expressions have been linked to many diseases, including cancer, endocrine disorders, and autoimmune diseases [10].

MiRNA-specific profiles were observed in PBMCs or serum from T1DM patients, and some miRNAs seem to modulate mRNA expressions of the major T1DM autoantigens [11]. On considering these aspects, the present study aimed to investigate the variable pattern of miRNA expression profiling in plasma obtained from patients with T1D and the matched control subjects through quantitative real-time PCR.

Methods

This case-control study was prospectively conducted on 90 children with T1D, with a mean age of (10.93 ± 4.51 years) having variable disease duration and variable degrees of glycemic control, who were diagnosed according to ADA criteria (Group I) [1].

A group of apparently healthy age and sex-matched 90 children were served as controls (Group II) with a mean age of (10.15 ± 2.56 years). All were enrolled from the Pediatric Department in collaboration with the Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Menoufia University Hospitals, Egypt. Collection of demographic data, anthropometric measurements, treatment regimens, and other clinical important parameters were done through viewing the medical sheet records for all sharing participants.

Cases who were suspected of not diagnosed as T1D; MODY, T2D, or secondary diabetes mellitus, or with evidence of chronic systemic/rheumatic diseases, inflammatory disorders, recent febrile illness, or on long-term steroid therapy were excluded from the study.

Upon approval of the study protocol from the Ethical Committee of Menoufia University per Helsinki II Declaration criteria, written informed consent was obtained from all participants in the study.

Following complete history taking & thorough clinical examination, all studied subjects underwent sampling of 7-10 ml whole blood after 12h overnight fasting via sterile techniques and divided into tubes as;

One ml of blood was transferred into sodium fluoride tube and another sample of blood was obtained after 2 hours for enzymatic colorimetric determination of blood glucose measured by the enzymatic colorimetric test, using Spinreact kit, SPAIN [12].

Another 4ml of blood was transferred into two EDTA tubes: one of them was used for quantitative colorimetric determination of glycated hemoglobin expressed as a percentage of the total hemoglobin by the use of kits supplemented by Teco diagnostics, USA [13]. Glycated hemoglobin (HbA1c) values $\geq 6.5\%$ were the limits for diagnosing T1DM [1].

The other EDTA tube was used for the extract of micro RNA molecules.

-For molecular analysis, 2ml of blood was transferred into one EDTA-containing tube and centrifuged for ten minutes at (4000) r.p.m. The clear supernatant was separated and kept frozen at -80°C until a further determination of MicroRNA levels.

RNA isolation

A total RNA including also miRNA molecules were purely extracted from plasma using Qiagen™ RNA Blood Mini Kit (Qiagen, USA, 2013) as defined by the manufacturer's instructions.

Reverse transcriptase PCR (RT-PCR):

RNA was reversely transcribed through Qiagen® miScript II RT Kit (Qiagen, Applied Biosystems, USA, 2012), reaching to 50µl reaction volume. The obtained complementary (c DNA) was then assayed with the universal SYBR Green Master Mix (QuantiTect SYBR Green PCR Kit, Qiagen).

For preparing RT Master Mix: 4 µl 5×miScript HiSpec Buffer, 2µl 10×miScript Nuclease Mix, 2 µl RNase-free water, 2 µl miScript Reverse Transcriptase Mix, then a 10 µl Template RNA

to reach a total reaction volume 20 µl. Reverse transcription was carried out at 37°C for 60 minutes and 95°C for 5 minutes on Applied Biosystems 2720 thermal cycler (Bioline, Singapore, USA) c DNA product was diluted to 5 ng/ul for the measurement of transcript levels by real-time quantitative PCR. Diluted c DNA was used as the template for real-time PCR with the miScript SYBR Green PCR Kit produced by Qiagen. Adding universal Primers was based on mRNA sequences delivered from the miR-Base database for (mi RNA 101-5p, mi RNA 146a-5p, mi RNA- 375, mi RNA 21-5p, miR 126, and miR

Let 7 a-5p) as shown in Table (1a). Each reaction for real-time PCR was completed to a final 25µL volume, as followed: 12.5 µl 2x QuantiTect SYBR Green PCR Master Mix, 2.5 µl 10x miScript specific Primer, 2.5 µl 10x miScript primer assay, 4 µl Template c DNA and 3.5 µl RNase-free water. the mixture was incubated at these conditions: 95°C for 15 min (as initial denaturation), then denaturation at 94°C for 15 s duration, annealing for 30 s at a temperature of 55°C and final extension for 30 s adjusted at 70°C, for designed 60 cycles. Amplification of small RNA RNU6B was performed with each experimental sample as an endogenous control. Data analysis was done in the real-time cycler Applied Biosystems® 7500 software version 2.0.1 thermal cycler (Applied Biosystems, Foster City, CA, USA).

The relative quantification (RQ) of genes expression was performed by comparative $\Delta\Delta C_t$ method, in which the amount of targeted mi RNAs 101-5p, mi RNA146a-5p, mi RNA- 375, mi RNA21-5p, mi RNA 126, and mi RNA Let 7 a-5p were normalized to RNU6B as an endogenous reference among patients and controls.

Table (1a): Primers used for the quantitative RT-q PCR assay for micro-RNA determination in all samples.

Mature miRNA symbol	Accession No.	Sequence	Catalogue No.
miR 101-5p	MIMAT0004513	5-CAGUUAUCACAGUGCUGAUGCU-3	MS00008379
miR 146 a-5p	MIMAT0000449	5-UGAGAACUGAAUCCAUGGGUU-3	MS00003535
miR 21 a-5p	MIMAT0004494	5-CAACACCAGUCGAUGGGCUGU-3	MS000009086
miR -375	MIMAT00000728	5-UUUGUUCGUUCGGCUCGUGA-3	MS000031829
miR 126 a-3p	MIMAT0000445	5-UCGUACCGUGAGUAAUAAUGCG-3	MS00003430
Let 7 a-5p	MIMAT0000062	5-UGAGGUAGUAGGUUGUACAGUU-3	MS00031220
RNU6B	Internal control	5-CGCAAGGATGACACGCAAATTCGTGAAGCGTTCCATATTTTT-3	MS00033740

Statistical analysis:

Data were analyzed using IBM SPSS statistics version 20 (SPSS Inc., Chicago, USA). Quantitative data were expressed as mean and standard deviation. A Chi-square test was used to examine the relation between qualitative variables. For quantitative data, a comparison between the two groups was done using either student t-test or Mann-Whitney test (non-parametric t-test) as appropriate. Spearman's correlation method was used to test the correlation between numerical variables. For the determination of T1D risk, multivariate logistic regression analysis was additionally used aided by the calculation of odds ratio (OR) and 95% Confidence Interval (CI). A p-value ≤ 0.05 was considered significant.

Results

In this study, a total of 180 children, including 90[46 males and 44 females] children with T1D (Group I), and healthy age and gender-matched 90[55 males and 35 females] control subjects (Group II) were investigated for the expression pattern for the circulating mi-RNAs- 101, 146, Let-7a, 21-5p, -375, and 126-5p molecules.

Their ages ranged from 10.93(4.51) for T1D patients and 10.15(2.56) for the control group. The demographic and clinical data of the studied groups were shown in **(Table 1)**.

Results of laboratory investigations for the group of T1D children including biochemical indices of; FBG, 2 h-PP, and mean HbA1C% were found to be statistically significant in comparison to controls (t-test: 23.985, 23.156 and 14.165, $P < 0.0001$) in order. All of the newly diagnosed cases with T1D disease duration, not more than 6-12 months were chosen as being positive for anti-insulin autoantibodies (IAAs), that ranged from 1 0-130 mIU/L

with mean value 10.42 ± 19.59 in those cases and from 0–7.0 mIU/L with mean value 2.08 ± 2.36 in healthy control children (U:7.03, P:0.002).

Comparative results regarding the levels of micro-RNAs studied in both groups revealed that miR-101-5p, miR-21-5p, miR 375 were highly expressed in patients with T1D, with a difference of statistical significance ($P < 0.05$), whereas miR146-5p, miR 126, and miR Let 7a-5p showed down-regulation of their plasma levels (p -value < 0.05) in order (**Table 2**). Of the remarkable findings in this study was that our results indicated a significant negative correlation of miR 101-5p with the age of onset ($r = -0.264$, $p = 0.015$) and with the duration of illness of T1D ($r = -0.162$, $p = 0.02$) in respect. MiR-146 was correlated with T1D disease duration ($r = 0.239$, $p = 0.023$). On the other hand, miR 126, and miR-Let7a-5p were significantly negatively correlated to mean T1D patients' glycated Hb A1c levels; p value < 0.05 (**Table 3**).

Results of multivariate logistic regression analysis for T1D risk were shown in (**Table 4**), where miR126-5p and miR-Let7a- markers showed highly significant findings after adjustment of values for age, sex and mean Hb A1C levels in patients group as evidenced by Odds ratio, CI 95% of 0.016(0.0–0.544), $p = 0.021$ for – 126-5p and 1.808(1.006–3.249) and p value = 0.048 for mi-Let7a- in order.

Correlations between mi-RNAs 101-5p, 126-5p, and certain diabetes parameters were shown in **Figs. 1, 2**.

The Amplification for micro-RNAs expression pattern (normalized fluorescent signal [ΔRn] that was plotted against the number of cycles) was evident in **figures from 3–8** in the corresponding sequence.

Discussion

The emerging role of miRNAs in modulating gene expression has greatly developed and is being recently implicated in the presentation of different diseases [14–16]. Validity Reliability in the level of expression of these circulating molecules favored the extreme ability to be recognized as key biomarkers of disease pathogenesis and progression status [17, 18]. This was evidenced by the relation of these molecules to 60% or more of the coding genes that thought to be in linkage to various endocrinal and autoimmune diseases [19–21].

Based on the underlying autoimmune background of T1D, our studied cases with recent disease onset; not more than 6–12 months duration; were chosen upon their positivity for insulin autoantibodies, the matter that researchers related certain miRNAs molecules to be validated as newly developed markers at early phases. Although for some, the progressive pattern in at-risk individuals couldn't be addressed, still several miRNAs are tightly associated with both glucose homeostasis and levels of autoantibodies to be the cornerstone in risk stratification [22].

In the issue of T1D, it's not still clearly understood whether the miRNAs have a cornerstone step in T1D pathogenesis or merely markers of active B cell dysfunctional outcome [23]. Butz et al reported a pivotal effect of mi- RNAs on pancreatic cellular biology, especially for B-cell differentiation, production of insulin, apoptosis, and mediation of inflammation [24].

In our study, among the analyzed mi RNAs, miR-146a-5p, Let-7a, and miR-126 were found to be down-regulated, whereas miR-101, 21-5p, and miR375a-5p were consistently up-regulated in patients when compared to our control subjects.

As for miR-375, its extreme abundance in pancreatic tissue rendered it a reflection of B-cell mass and alterations in its metabolic functions [25]. In respect to our results, it was found that the level of miR-375 in the plasma of patients with T1D was significantly increased. When globally analyzed for its correlation to HbA1C, no difference was noticed as evidenced by a coefficient r-value of 0.173 and a p-value of 0.201. The relation of that molecule to meanA1C values was favored by Marchand et al., 2016 who found dysregulated miR-375 level in the blood of newly diagnosed children with type 1 diabetes when quantified to high levels in human islet tissue, the matter that conferred that to be a hallmark in the etiology of T1D. Furthermore, it may be a marker of the early phases of the disease [26].

Of the deregulated molecules in our study that was found to be up-regulated in the group of T1D patients versus those of control subjects as a difference of statistical significance (p value < 0.001, Table 2) was 21-5p, which was examined by Pan et al., for the involvement of enteroviral infection on 21-5p expression and subsequent contribution to T1D [27].

Analyzing data from past literature, revealed that has-miR-21 seemed to be highly expressed in plasma of T1D patients in comparison to controls. Osipova et al., 2014 [28] found similar results. Ongoing research related 21-5p to cytokines of inflammation. In addition to the findings of Backe and coworkers [29], it was suggested that miR-21 overexpression was believed to influence the Bax group/ apoptotic signaling pathway, hence inducing pancreatic B-cell death. This process could be served as a new therapeutically tried target for T1DM [30, 31].

Another up-regulated miRNA in our study was – 101-5p, that targeted reduction of insulin secretion and B-cell mass as a favor of its involvement in cytokine release regulation and altered signaling of STAT3, HGF/C-Met and Ephrin receptors pathway mechanisms. Adjuvant to our findings of the significant association of miR101-5p to insulin autoantibody-positive cases of recent onset T1D with disease duration, not more than 6–12 months, this matter that was largely analyzed by Santos A et al., who reported that the expression of miR-101 was about 3fold higher in patients with multiple autoantibodies level[32].

As found in our study, the negative correlation of miR-101 with the age of onset of T1D ($r = -0.264$, $p = 0.015$), related studies suggested a greater rate of B-cell turnover and pancreatic injury in young children with T1DM [33, 34].

Another important micro-RNA molecule that showed significant down regulation besides being indicated in patients with recent-onset T1D was the miR146 a-5p. This was evident through a lowered expression level in cases; median (IQR) of 0.16(0.02–0.43) compared to levels of 0.77(0.0–0.88) in control subjects ($p < 0.001$). A possible biological effect explored from its consistent relation to genes linked to apoptotic and innate immune regulatory pathway mechanisms [35].

Let7 a-5p was one of the studied markers expressed at lower levels in the studied group of patients and demonstrated a statistically significant difference in comparison to that of controls. Similar results were evident in the study done by Tian C et al., where Let – 7a was down-regulated in both human and mice tissue derivatives [36]. The later was known to be involved in the regulation of glucose metabolism. In agreement with our results, it was found to be negatively correlated to A1C by the study done by Erener et al., 2017 [37].

Assessing the level of miR-126 expression revealed contradictory findings. Osipova et al., conducted lower urinary levels in patients with T1D, with no significant difference in plasma samples of the studied cases and controls respectively [28]. However, the observation of Wang et al. clarified decreased plasma levels of miR-126 in those

with chronic ESRD [38]. Despite the disagreeable findings identified in our study about the significant lower plasma level of the miR-126 in T1D patients to that of Osipova et al., they came in the same line with the proposed mechanism that related decreased level of miR-126 to deranged response to vascular endothelial growth factor (VEGF) and endothelial dysfunction [28, 39, 40]. Also, previous reports considered this marker as a controlling factor for various biological processes [41, 43], through linkage of decreased circulatory miR-126 levels to micro-vascular change and possibility of later-on long-standing T1DM complications [43]. The noticed significant negative correlation between miR-126, mi-R Let7a- markers, and high percentages of the mean HbA1C values suggested the significant association of hyperglycemia to of the altered levels of the circulating miRNAs to hyperglycemic state [37]. These above considerations were nearly similar to the hypothesis of Akerman et al., 2018 who assumed that expression profiling of miRNAs may be of value regarding their feasibility to be a distinguishing complementary marker in risky individuals with abnormal OGTT results [22].

Conclusion

Deregulation of microRNAs in our study revealed down-regulation of miRNAs 146-5p, 126-5p and Let 7a-5p molecules and the up-regulation of miR101-5p, 21-5p and 375- were identified in our study for their significant relation to T1DM. The additional significant negative correlation of miR126- 5p and Let7a-5p micro RNAs with mean glycated HbA1C values are indicative of possible use as biomarkers hyperglycemia-associated pathophysiologic changes in T1DM.

Given the stability, reliability of these markers, they were preferred for their superiority over other quantification techniques establishment through q-RT-PCR and warranted our choice in that study to be the **1st** estimate for micro-RNA profiling among Egyptian children having type1diabete, the matter that potentiates pavement of the way to their targeted usage as new intervention therapeutic markers. Of course, further larger-scale functional studies are required for genetic interactions thus improving the quality and life expectancy of children with T1DM.

Abbreviations

CI

Confidence Interval.

CVD

Cardiovascular diseases

ESRD

End-stage renal disease

HbA1c

Hemoglobin A1C.

Mi RNA

Micro ribonucleic acid.

OGTT

Oral glucose tolerance test.

OR

Odds ratio.

PBMCs

Peripheral blood mononuclear cells.

PCR

Polymerase chain reaction.

Q-RT-PCR

Quantification techniques- Polymerase chain reaction.

RQ

Relative quantification

RT

Reverse transcriptase.

T1DM

Type 1 diabetes mellitus.

UTRs

Untranslated regions.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

NFB designed the study, analyzed data and drafted the manuscript MMM and EMA participated in the design of the study and coordination of the whole work. NFB and MMM collected and organized patients' data. NFB and EMA performed molecular genetic studies for patients. NFB and MMM analyzed the data. All authors have read and approved the manuscript and ensure that this is the case.

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Availability of data and material:

The datasets are available from the corresponding author on reasonable request.

Ethics approval and consent to participate:

All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional review committee of Huang et al. BMC Endocrine Disorders (2020) 20:99 Page 7 of 9 the Third Affiliated Hospital of Soochow University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The present study was approved by the Institutional Review Committee of the Faculty of Medicine, Menoufia University.

All subjects gave written, informed consent through their parent or guardian before enrollment in the study.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

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Tables

Table (1): Comparison between the two studied group as regarding to demographic, clinical and laboratory data.

	T1DM (n = 90)		Control (n = 90)		Test of sig.	P
	No.	%	No.	%		
Age (years)	46 44	51.1 48.9	55 35	61.1 38.9	$\chi^2=1.827$	0.176
Sex (male/female)	48 52	48 52	65 35	65 35	2.9	0.006
Family history (positive/negative)	21 79	21 79	0 100	0 100	20.1*	<0.001*
Duration (years) mean $\pm$ SD.	10.93 $\pm$ 4.51		10.15 $\pm$ 2.56		t=1.434	0.154
Systolic blood pressure(mm/hg) mean $\pm$ SD.	107.4 $\pm$ 6.10		110.0 $\pm$ 4.50		t=3.199*	0.002*
Diastolic blood pressure(mm/hg) mean $\pm$ SD.	67.78 $\pm$ 5.95		67.0 $\pm$ 6.44		t=0.841	0.401
Weight (KG/M2) mean(SD) range	19.6 $\pm$ 3.98 13-29		20.22 $\pm$ 5.51 13.3-32.5		t=0.91	0.32
Time from onset mean $\pm$ SD. mean (IQR)	6.93 $\pm$ 3.29 7.0(5.0 - 10.0)					
Duration of illness mean $\pm$ SD. mean (IQR)	4.41 $\pm$ 3.35 4.42(1.0 - 7.0)					
Diagnosis (typical/atypical)	30 60	33.3 66.7				
Complications (C%)					t=	<0.001*

Mean ± SD.	9.03 ± 2.04		5.94 ± 0.34		14.165*	
Median (IQR)	9.0(7.2 – 10.0)		5.95(5.7 – 6.2)			
ng/dl)						
Min. – Max.	105.0 – 300.0		75.0 – 105.0		t=	<0.001*
Mean ± SD.	210.52 ± 46.46		91.0 ± 8.76		23.985*	
Median (IQR)	200.0(180.0 – 250.0)		91.0(85.0 – 98.0)			
P(mg/dl)						
Min. – Max.	130.0 – 310.0		140.0 – 172.0		t=	<0.001*
Mean ± SD.	257.11 ± 40.31		154.80 ± 11.47		23.156*	
Median (IQR)	260.0(220.0 – 300.0)		155.5(145.0 – 165.0)			
Microalbuminuria						
	82	91.1	91	100.0	$\chi^2=$	FE _p =
	8	8.9	0	0.0	8.372	0.007*
Min. – Max.	206.20 – 394.0		-		-	-
Mean ± SD.	265.55 ± 80.19		-		-	-
Median (IQR)	231.0(215.1 – 316.0)		-		-	-

χ^2 : Chi square test FE: Fisher Exact MC: Monte Carlo IQR: Inter-quartile range

t: Student t-test U: Mann Whitney test p: p value for comparing between the studied groups *:

Statistically significant at $p \leq 0.05$.

Table (2): Comparison between the two studied groups according to different microRNAs expression.

	T1DM (n = 90)	Control (n = 90)	U	P
microRNAs expressions				
microRNA101-5p				
Min. - Max.	0.0 - 1640.55	0.09 - 1.66	3652.0	0.028*
Mean $\pm$ SD.	65.33 $\pm$ 270.39	0.39 $\pm$ 0.14		
Median (IQR)	0.60(0.08 - 1.95)	0.26(0.10 - 0.35)		
microRNA146a-5b				
Min. - Max.	0.0 - 328.49	0.0 - 1.21	2646.0*	<0.001*
Mean $\pm$ SD.	7.89 $\pm$ 48.63	0.63 $\pm$ 0.44		
Median (IQR)	0.16(0.02 - 0.43)	0.77(0.0 - 0.88)		
microRNA 375a-3p				
Min. - Max.	0.0 - 2127.09	0.07 - 2.93	1908.0*	<0.001*
Mean $\pm$ SD.	70.36 $\pm$ 332.53	0.95 $\pm$ 1.02		
Median (IQR)	2.10(1.08 - 3.11)	0.53(0.09 - 1.05)		
microRNA 21-3p				
Min. - Max.	0.0 - 89.39	0.0 - 0.06	2682.0*	<0.001*
Mean $\pm$ SD.	2.27 $\pm$ 13.21	0.03 $\pm$ 0.02		
Median (IQR)	0.08(0.0 - 0.48)	0.02(0.0 - 0.05)		
microRNA -126				
Min. - Max.	0.00 - 0.62	0.00 - 152.32	1569.50*	<0.001*
Mean $\pm$ SD.	0.19 $\pm$ 0.15	4.50 $\pm$ 22.39		
Median (IQR)	0.15(0.07 - 0.31)	0.69(0.26 - 1.61)		
microRNA -Let 7a-5p				
Min. - Max.	0.0 - 35.80	0.0 - 1.92	2356.50*	<0.001*
Mean $\pm$ SD.	0.94 $\pm$ 5.21	0.50 $\pm$ 0.51		
Median (IQR)	0.09(0.02 - 0.23)	0.12(0.10 - 1.02)		

U: Mann Whitney test p: p value for comparing between the studied groups *: Statistically significant at $p \leq 0.05$

Table (3): Correlation between different parameters and all microRNAs in patients with T1D.

		microRNA 101-5p	microRNA 146a-5b	microRNA 375a-5p	microRNA 21-3p	microRNA -126	microRNA -Let 7a-5p
Duration of illness	r_s	-0.162	0.239	0.095	-0.151	-0.167	-0.034
	P	0.02*	0.023*	0.386	0.167	0.128	0.756
Age of onset	r_s	-0.264	0.149	-0.008	0.211*	0.089	-0.062
	P	0.015*	0.160	0.943	0.046*	0.402	0.563
HbA1c	r_s	0.090	0.183	0.173	0.054	-0.214	-0.216
	P	0.413	0.162	0.201	0.610	0.042*	0.043*
FBS	r_s	-0.194	0.176	-0.147	0.166	-0.032	0.241
	P	0.067	0.097	0.166	0.119	0.764	0.022
2hr pp	r_s	-0.215	0.134	-0.008	0.096	0.006	0.123
	P	0.042	0.209	0.939	0.368	0.954	0.247

r_s : Spearman coefficient *: Statistically significant at $p \leq 0.05$

Table (4): Univariate and multivariate analysis for the parameters affecting T1DM risk (No. = 90)

	Univariate		Adjust Odd`s ratio	
	P	COR (95%C.I)	p	AOR# (95%C.I)
microRNA 101-5p	0.009*	1.565(1.121 - 2.185)	0.307	1.783(0.587 - 5.414)
microRNA 146a-5p	0.616	1.017(0.951 - 1.088)	0.844	1.009(0.924 - 1.101)
microRNA Let 7 a-5p	<0.001*	1.845(1.370 - 2.483)	0.048*	1.808(1.006 - 3.249)
microRNA 21-5p	<0.001*	11.62 (3.63.69 - 71.85)	0.026*	7.180(2.554 - 20.187)
microRNA -126	<0.001*	0.013(0.002 - 0.064)	0.021*	0.016(0.0 - 0.544)
microRNA -375	0.453	1.037(0.943 - 1.140)	0.921	1.014(0.776 - 1.324)

OR: Odd`s ratio, C.I: Confidence interval, AOR#: adjust Odd`s ratio by family history and HbA1C

*: Statistically significant at $p \leq 0.05$.

Figures

Figure 1

Correlation results between microRNA 101-5p with age of T1D onset.

Figure 2

Correlation results between microRNA -126 and HbA1c.

Figure 3

Amplification plot for mi RNA 21-5p expression pattern (normalized fluorescent signal ΔR_n plotted against the number of the cycle).

Figure 4

Amplification for mi RNA 101-5p expression (normalized fluorescent signal [ΔRn] plotted against the number of the cycle).

Figure 5

Amplification for mi RNA 146a-5p expression (normalized fluorescent signal [ΔRn] plotted against the number of the cycle).

Amplification Plot

Figure 6

Amplification for mi RNA- 375 expression (normalized fluorescent signal [ΔRn] plotted against the number of the cycle).

Amplification Plot

Figure 7

Amplification plot of miR Let 7 a-5p expression (normalized fluorescent signal [ΔRn] against the number of the cycle).

Figure 8

Amplification of miR 126 gene expression (normalized fluorescent signal [ΔRn] against the number of the cycle).