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RESEARCH ARTICLE

The possible role of Micro RNA-451 in children with Type 1 Diabetes Mellitus

Mahmoud Mohamed Abd Elkhalek Hodeib[1], Osama Ezzat Botrous[1], Emad Eldeen Ameen Abdulkader[1]*, Mahmoud Mahmoud Noureldeen[1]
[1]Pediatrics department, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

*Corresponding Author
Emad Eldeen Ameen Abdulkader

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Abstract: ***Background:*** Type 1 diabetes (T1DM) is a multifactorial condition that causes organ-specific autoimmune destruction of pancreatic insulin-producing beta cells in HLA-positive individuals. Micro RNA (miR)-451 is synthesized by immune cells and may modulate their own actions as well as those of other immune cells. ***Objectives:*** to assess the possible role of miR-451 in children suffering from T1DM. ***Methods:*** 50 children with T1DM and 20 healthy age and sex matched controls were enrolled in this study. After basic history and examination of participants, blood and urine samples were obtained from each subject to perform complete blood picture (CBC), Random blood sugar (RBS), and HBA1C. Reverse transcriptase real time PCR was used to estimate miR -451 in blood samples of cases and controls. ***Results:*** miRNA-451 gene expression was significantly higher in cases (1.84 ± 0.86) than controls (1.14 ± 0.55) with (P value <0.001). MiR-451 gene expression was significantly and positively correlated with age (P=0.004), height (P=0.006), RBS (P=0.004), BW (P=0.026), and HgA1C (P=0.007 In the current study; miRNA- 451 gene expression was significantly higher in cases (1.84 ± 0.86) than controls (1.14 ± 0.55) with (P value <0.001). MiR-451 gene expression was excellent in diagnosing T1DM with AUC = 0.734. Using >1.585 as a cut off value both sensitivity and specificity of miRNA- 451 gene expression in diagnosing T1DM were 64% and 80% respectively. Multivariate logistic regression to detect independent predictors of T1DM showed that; micro RNA 451 and age are independent predictors for T1DM in children. Linear regression to detect if micro RNA 451 acts as independent predictors of HbA1c showed that; micro RNA 451 acts as independent predictors of HbA1c. ***Conclusion:*** MiRNA-451 is upregulated in T1DM patients and could be used as biomarkers for the disease. These results could be a starting point for future research on these microRNAs as new biomarkers for T1DM.

Keywords: Type 1 diabetes, autoimmune diseases, MiR -451.

INTRODUCTION

The autoimmune death of insulin-producing pancreatic beta cells in those genetically predisposed by human leukocyte antigen (HLA) is a hallmark of T1DM. This condition is one of many contributing factors. Approximately 5–10% of people with diabetes have T1DM. A malfunction in immunological regulation leads to the development of T1DM by triggering the innate immune system to activate, the proliferation of autoreactive CD4+ and CD8+ T cells, and B lymphocytes that secrete autoantibodies [1]. Insulitis, a chronic inflammatory infiltration into the pancreatic islets, is the principal histological finding in T1DM. The inability of the remaining pancreatic β-cells to recover after autoimmune damage is another important consideration in persons suffering from chronic disease [2].

MicroRNAs (miRNAs) are endogenous tiny RNA molecules that regulate gene expression post-transcriptionally. Due to their widespread distribution in tissues and fluids, these molecules are essential to biological activities. MiRNAs are important in fundamental biological research and clinical translational applications because they may be disease biomarkers [3, 4].

Altuvia et al. [5] found the human pituitary gland gene encoding miR-451 on the 17q11.2 chromosome in 2005. This groundbreaking finding has elevated miR-451 to the forefront of scientific inquiry, highlighting its importance in several physiological and pathological roles.

MiR-451 modulates several immune cells, which affects disease development, according to subsequent studies. MiR-451 regulates microglia, macrophages, and neutrophils [6–8].

The current study was to assess the possible role of miR-451 in children suffering from T1DM.

2. Patients and methods

This case control study was conducted in Pediatric Departments at Beni-Suef University Hospital. 50 patients with T1DM and 20 healthy age and sex matched controls.

Study Participants:

Participants were selected randomly and were included in the study if; Age < 18years males or females with known T1DM and who are receiving their regular and routine antidiabetic insulin therapy. Participants were excluded from the current study if they had one of the

following: endocrinal diseases other than DM, or auto immune diseases other than T1DM.

Type 1 DM was diagnosed based on the 2016 American Diabetes Association diagnostic criteria for diabetes mellitus **[9]**.

MATERIAL AND METHODS

All patients were subjected to the following:
History taking with special focus on history of DKA, family history of diabetes, type of insulin used, recurrent Urinary tract infections, hematuria, urine output, and hospital admissions. Weight, height BMI and ambulatory blood pressure monitoring.

The performed investigation in all participants were; CBC with differential, Random blood sugar (RBS), HBA1C and gene expression of miRNA-451.

Blood Samples
A ten-milliliter blood sample was obtained from each subject after eight hours of fasting and divided into three aliquots. One aliquot was used for complete blood counts by Sysmex system and HBA1c by Stanbio kit (Boerne, Texas, USA, Cat. No. 0350) by the use of quantitative colorimetric method using column chromatography for determination of glycohemoglobin in whole blood. From the other aliquot, serum was separated and subjected to laboratory measurement of fasting blood glucose level by autoanalyzer Dialab (A-2351 Wr. Neudorf, Austria). The serum was separated from the third aliquot and kept frozen at −80°C for the molecular study of miRNA-451 by a real-time polymerase chain reaction.

Gene expression of miRNA-451 measurement:
Gene expression of miRNA-451 was assessed by reverse transcriptase real time PCR.

- RNA isolation

Human peripheral blood mononuclear cells were extracted from whole blood using RNeasy® UCP MinElute® spin columns (Quiagen, USA, cat. no. 217204). The detailed method was according to the manufacturer's protocol.

- Quantitative real-time PCR (qRT-PCR)

RNA was subjected to reverse transcription reactions by using the All-in-One™ miRNA qRT-PCR Detection Kit 2.0 (GeneCopoeia, Inc, Rockville, USA, Cat. No. QP115) according to manufacturer instructions. Relative fold changes of gene expression were calculated by the DDCT method and the values were expressed as 2DDCt. All experiments were performed at least three times.

- MiR-451 PRIMER:

F: 5′- GGA AGA TCT TGA CAA GGA GGA CAG GAG AG -3′
R: 5′- CCC AAG CTT GCC TTG TTT GAG CTG GAG TC -3′

Ethical considerations:
The parent(s) for all children included in the study were informed about the procedures regarding the study and were informed of their rights to refuse their children participation or withdraw from the study without having to give reasons. Children were guaranteed anonymity and all information provided was treated with confidentiality. The ethical approval of the faculty of medicine, Beni-Suef University research ethical committee (REC) was obtained prior to the beginning of the work. Approval number is ().

Statistical analysis:
Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Data was double checked for normality using normality plots and Shapiro Wilk test. Comparisons between groups were done using unpaired t test in normally distributed quantitative variables while non-parametric Kruskal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient. ROC curve was constructed with area under curve (AUC) analysis performed to detect best cutoff value of micro RNA 451 for detection of DM. P-values less than 0.05 were considered as statistically significant**.**

RESULTS AND OBSERVATIONS:

In table (1): There are significant differences between cases and controls as regard age, the mean age was (11.02 ± 3.37) years in cases while in controls was (7.60± 2.58) years with (p value 0.0001); height the mean height was (141.98± 18.44) cm in cases while in controls was (124.40± 14.93) cm with (p value 0.0001) and BW the mean BW was (32.04 ± 14.80) kg in cases while in controls was (25.00± 8.30) kg with p value (0.010). While There are significant differences between cases and controls as regard BMI (kg/m2), the mean BMI was (14.64± 3.77) (kg/m2) in cases while in controls was (15.53± 1.22) (kg/m2) with (p value 0.13)

Table (1): Comparison between cases and controls regarding age and anthropometric measurements

	Cases (n=50)	Controls (n=20)	P
Age(years)	11.02 ± 3.37	7.60± 2.58	0.00
BW (Kg)	32.04 ± 14.80	25.00± 8.30	0.01
HEIGHT (cm)	141.98± 18.44	124.40± 14.93	0.00
BMI (kg/m2)	14.64± 3.77	15.53± 1.22	0.13

In table (2): RBS was higher in cases (201.38 ± 60.02) mg/dL than controls (95.80± 7.54) mg/dL with p value (<0.001). FBS was higher in cases (110.64± 16.88) mg/dL than controls (97.87± 7.38) mg/dL with p value (<0.001). Hb A1c was higher in cases (8.42± 2.27) % than controls (4.67± 0.48) % with p value (<0.001).

Table2: Comparison between cases and controls as regarding glycemic control

	Cases (n=50)	Controls (n=20)	P
RBS mg/dL	201.38 ± 60.02	95.80± 7.54	0.00
FBS mg/dL	110.64± 16.88	97.87± 7.38	0.00
HGA1C %	8.42± 2.27	4.67± 0.48	0.00

In table (3): No significant differences between cases and controls as regard HB, the mean HB was (11.18 ± 0.83) g/L in cases while in controls was (11.40± 0.72) g/L with (p value 0.17); TLC the mean TLC was (6756.20± 1421.52) x 10^9/L in cases while in controls was (6515.07± 782.06) x 10^9/L with (p value 0.32) and PLT the mean PLT was (359.40± 93.52) x 10^9/L in cases while in controls was (374.67± 93.66) x 10^9/L with (p value 0.43).

Table (3): Comparison between cases and controls as regarding CBC data

	Cases (n=50)	Controls (n=20)	P
HB g/L	11.18 ± 0.83	11.40± 0.72	0.17
TLC x 10^9/L	6756.20± 1421.52	6515.07± 782.06	0.32
PLT x 10^9/L	359.40± 93.52	374.67± 93.66	0.43

In table (4) and figure (1): miRNA- 451 gene expression was significantly higher in cases (1.84 ± 0.86) than controls (1.14 ± 0.55) with (P value <0.001).

Table (4): Comparison between cases and controls as regarding miRNA-451 levels

	Cases (n=50)	Controls (n=45)	P
mRNA-451	1.84 ± 0.86	1.14 ± 0.55	0.00

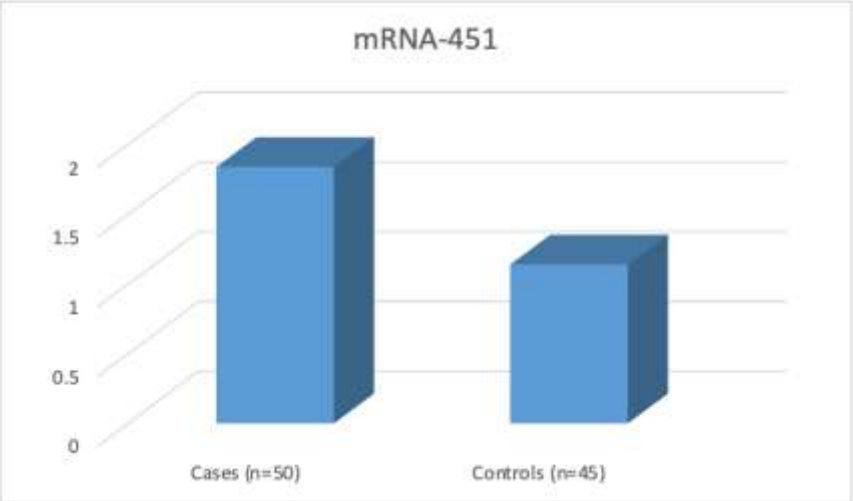

Fig1: Comparison between cases and controls as regarding miRNA-451 levels

In table (5): miR- 451 gene expression was significantly and positively correlated with age (p value .004), height (p value .006), RBS (P value .004), BW (P value .026), and HgA1C (P value .007). miR-451 gene expression was not significantly correlated with BMI (P value .026), FBS (P value .061), HB (P value .311), TLC (P value .731), and PLT (P value .143).

Table (5): Correlations between miR-451 levels and study variables

		miR-451
Age (years)	Pearson Correlation	.290**
	Sig. (2-tailed)	.004
BW (kg)	Pearson Correlation	.229*
	Sig. (2-tailed)	.026
HEIGHT (cm)	Pearson Correlation	.278**
	Sig. (2-tailed)	.006
BMI (kg/m2)	Pearson Correlation	-.037
	Sig. (2-tailed)	.718
RBS (mg/dL)	Pearson Correlation	.293**
	Sig. (2-tailed)	.004
FBS (mg/dL)	Pearson Correlation	.302**
	Sig. (2-tailed)	.061
HGA1C (%)	Pearson Correlation	.277**
	Sig. (2-tailed)	.007
HB (g/L)	Pearson Correlation	-.105
	Sig. (2-tailed)	.311
TLC (10^9/L)	Pearson Correlation	-.036
	Sig. (2-tailed)	.731
PLT (10^9/L)	Pearson Correlation	-.151
	Sig. (2-tailed)	.143

As shown in table (6) and figure (2): MiR-451 gene expression was excellent in diagnosing T1DM with Area under the ROC curve = 0.734. Using >1.585 as a cut off value both sensitivity and specificity of miRNA- 451 gene expression in diagnosing T1DM were 64% and 80% respectively.

Table (6): The cutoff value that can discriminate between cases of T1DM and controls using micro RNA 451

Area Under the Curve	P value	95% Confidence Interval		Cut off	Sensitivity %	Specificity %
		Lower Bound	Upper Bound			
0.734	< 0.001	0.636	0.832	1.585	64	80

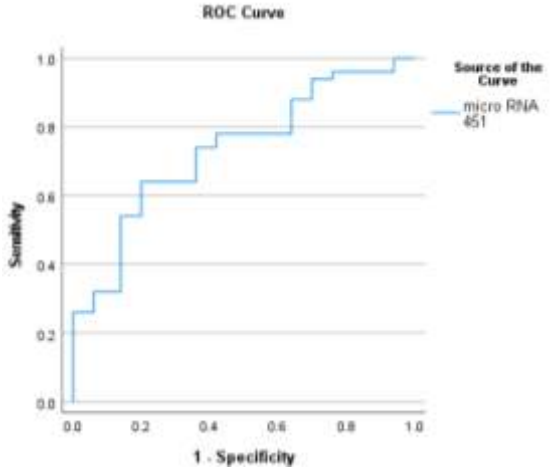

Figure (2): ROC curve for prediction of T1DM using micro RNA 451

DISCUSSION

It is possible that an explanation of the disease's mechanisms could be obtained by identifying new biomarkers and determining their connections to the pathophysiology of T1DM. Hopeful markers may empower earlier T1DM diagnosis and better care for T1DM patients, enhancing the quality of life [10].

Overall, miR-451 has emerged as a promising diagnostic biomarker across multiple pathological conditions, with its expression patterns showing distinct characteristics based on disease types and sample origins. Subsequent investigations have elucidated miR-451's capacity to modulate an array of immune cells, thus playing a consequential role in disease progression. Specifically, miR-451's regulatory influence spans across several immune cell types, including but not limited to Lymphocytes, microglia, macrophages, and neutrophils [11-13].

The current study was to assess the possible role of miR-451 in children suffering from T1DM. This case control study was conducted in Pediatric department at Beni-Suef University Hospital. 50 patients with diabetes and 20 healthy age and sex matched controls. There are significant differences between cases and controls as regard age, height, BW and BMI (p value 0.13). RBS was higher in cases (201.38 ± 60.02) mg/dL than controls (95.80± 7.54) mg/dL with p value (<0.001). FBS was higher in cases (110.64± 16.88) mg/dL than controls (97.87± 7.38) mg/dL with p value (<0.001). Hb A1c was higher in cases (8.42± 2.27) % than controls (4.67± 0.48) % with p value (<0.001).
No significant differences between cases and controls as regard HB, TLC and PLT.

Similarly; laboratory investigations in Abbas et al., [14] of the studied groups clarified that FBS, 2 hour postprandial sugar, and HbA1c% were significantly higher in the T1DM group compared to healthy controls. In line with our results, Abdelsalam et al. found that when compared to controls, diabetic patients had significantly greater FBS and HbA1c (P < 0.001) [15]. Also, Assmann et al. reported that T1DM patients had higher mean HbA1c levels than healthy controls (P < 0.001) [16]. Dieter et al. [17] showed that when compared to controls, HbA1c levels were elevated in diabetic groups (moderate and severe) (P = 0.005).

Also in the study of Adam et al., [18] the WBCs count was elevated in DM. lead to a decrease or prevention of insulin production, which result in a group of metabolic imbalances accompanied by multiple disorders in the metabolism of lipid, carbohydrate, and protein [19]. DM is accompanied by hyperglycemia, hyperlipidemia, and glycosuria. Hyperglycemia is responsible for multiple physiological defects such as vasodilation, inflammatory regulation, immunological indices, hematological indices (size, morphology, and function of WBCs, RBCs, and platelets), and cell growth in uncontrolled diabetes [20].

In the current study; miRNA- 451 gene expression was significantly higher in cases (1.84 ± 0.86) than controls (1.14 ± 0.55) with (P value <0.001). MiR-451 gene expression was excellent in diagnosing T1DM with AUC = 0.734. Using >1.585 as a cut off value both sensitivity and specificity of miRNA- 451 gene expression in diagnosing T1DM were 64% and 80% respectively.

In the same context; Abbas et al., [21] reported that miRNA-451 was significantly upregulated in T1DM compared to controls (p < 0.001). This result is consistent with Collares et al., [22] who found that miR-451 was upregulated in T1DM versus T2DM . According to Abdelsalam et al. study [15], the sensitivity of plasma miRNA-451 was 90.9% at a cutoff point of 27.5 with a specificity of 67.7%. In comparison, the sensitivity of plasma miRNA-451 was 95.5% at a cutoff point of 19.5 with a specificity of 95.6% .

In the current study; miR- 451 gene expression was significantly and positively correlated with age, height, RBS, and HgA1C.

Similarly; Abbas et al., [21] reported a significant positive correlation between miRNA-30 and FBS and between miRNA-451 and both FBS, 2hPPBS, HbA1c, and disease duration. At the same time, there was a significant negative correlation between miRNA-451 and the child's age and the age of the onset of T1DM.

According to their HbA1c values, all the T1DM patients in the current study had inadequate glycemic control. Consequently, our findings may be attributable to a relationship with hyperglycemia and not autoimmunity and beta-cell death. MiRNA-451 expression was negatively connected with HbA1c levels in the T1DM group, suggesting it is associated with hyperglycemia. This miRNA also participate in the immune system and apoptosis-related beta-cell destruction pathways. Erener et al. [23] also observed miRNAs linked with hyperglycemia in early-stage T1DM serum.

A malfunction in immunological regulation leads to the development of T1DM by triggering the innate immune system to activate, the proliferation of autoreactive CD4+ and CD8+ T cells, and B lymphocytes that secrete autoantibodies [1]. MiR-451 inhibits the proliferation of CD4+ T cells following malaria infection in mice. Also prior findings lend support to the potential of miR-451 as a biomarker for T cell and B-cell-related diseases [24, 25].

Overall, miR-451 has emerged as a promising diagnostic biomarker across multiple pathological

conditions. In systemic inflammatory and autoimmune disorders, including rheumatoid arthritis, Hashimoto thyroiditis, and systemic lupus erythematosus, circulating miR-451 levels are predominantly upregulated [26-28].

CONCLSION

MiRNA-451 is upregulated in T1DM patients and could be used as biomarkers for the disease. These results could be a starting point for future research on these microRNAs as new biomarkers for T1DM. However, further comprehensive research is needed to explore additional interactions between miR-451 with T and B cells beyond its biomarker function to be used in therapeutic function.

6. Conflict of interest:
Nil

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