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Serum miRNA-146 and Cortisol levels in Type 2 Diabetes Mellitus: Novel Biomarkers for Diagnosis and Pathophysiological Insights

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Abstract

Background: Type 2 Diabetes Mellitus (T2DM) is a chronic condition characterized by hyperglycemia due to defects in insulin action or secretion. Its rising prevalence poses a significant global health challenge. Evidence suggests that T2DM involves non-coding RNA molecules, such as miRNAs, which regulate gene expression. Cortisol, a stress hormone, is also implicated in T2DM by influencing glucose metabolism and contributing to complications. Aim: This study aimed to evaluate the diagnostic and pathophysiological relevance of miRNA-146 and cortisol in T2DM. This study explored their potential as biomarkers for identifying metabolic and inflammatory abnormalities and examined their interactions with glycemic markers (HbA1c and RBG). Methods: This cross-sectional study included 100 T2DM patients and 100 healthy controls from the Suez Canal University outpatient clinic. Biochemical assessments included RBG, fasting insulin, HbA1c, vitamin D, cholesterol, triglycerides (TG), LDL, HDL, cortisol, and miRNA-146. Cortisol and vitamin D levels were measured using ELISA, while miRNA-146 levels were quantified using RT-qPCR. Diagnostic performance was assessed using Receiver Operating Characteristic (ROC) curves, and statistical analyses were performed using IBM SPSS. Results: miRNA-146 levels were significantly lower in T2DM patients than in controls, with a cut-off value of ≤ 6.83, achieving 83.0% sensitivity and 79.0% specificity (AUC = 0.832). This makes miRNA-146 a highly effective diagnostic biomarker for early detection. Cortisol levels were elevated in T2DM patients and positively correlated with HbA1c and RBG, indicating its role in glucose metabolism dysregulation. A cortisol cut-off of ≥ 3.8 nmol/L showed 95.0% sensitivity and 71.0% specificity (AUC = 0.819), suggesting its utility as a secondary screening tool. However, its lower specificity may warrant further confirmatory testing. Conclusion: This study highlights the complementary roles of miRNA-146 and cortisol as T2DM biomarkers. While miRNA-146 demonstrates the highest diagnostic accuracy, cortisol offers additional insights into metabolic disturbances. These findings provide a foundation for improving T2DM diagnosis and monitoring. Further research is needed to validate these biomarkers and to explore their potential as targeted therapeutic strategies.

Keywords: Type 2 diabetes mellitus; MiRNA-146; Cortisol; HBA1c; Biomarker, hyperglycemia

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It encompasses two primary types:

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Type 1 Diabetes Mellitus (T1DM), which results from insufficient insulin production by pancreatic β-cells, and Type 2 Diabetes Mellitus (T2DM), the most common form, which is primarily caused by insulin resistance and impaired β-cell function [1,2].

Globally, T2DM is a leading cause of mortality and morbidity, ranking third after cardiovascular diseases and cancer [3]. In 2019, over 463 million individuals were diagnosed with diabetes, and 90% of these cases were attributed to T2DM. This prevalence is projected to increase to 10.9% by 2045, highlighting the urgent need for improved preventive and diagnostic strategies [4].

The pathogenesis of T2DM is multifactorial and involves complex interactions between genetic predispositions and environmental factors such as age, family history, obesity, and dietary habits. Genome-wide association studies have identified over 400 gene loci associated with increased susceptibility to T2DM, highlighting the significant contribution of hereditary variations to disease development [5]. However, environmental factors and their interaction with genetic predispositions further complicate T2DM's progression.

Recently, microRNAs (miRNAs) have emerged as promising biomarkers for the early detection and understanding of T2DM pathogenesis. miRNAs are non-coding RNAs that post-transcriptionally regulate gene expression by targeting specific mRNAs. Of the 2,588 known human miRNAs, nearly half of all mammalian protein-coding genes are regulated by miRNAs, highlighting their extensive influence on biological processes [6, 7]. Their tissue-specific expression and role in regulating metabolic pathways, including glucose homeostasis, make miR-NAs key players in T2DM [8, 9]. Among these, miRNA-146 is particularly notable for its role in modulating the inflammatory responses. miRNA-146 acts as a negative regulator of inflammatory pathways by inhibiting genes involved in the nuclear factor-kappa B (NF-kB) signaling cascade. NF-kB, a key pro-inflammatory transcription factor, stimulates miRNA-146 expression, forming a feedback loop that attenuates inflammation. However, reduced miRNA-146 levels have been linked to chronic inflammation, a major contributor to T2DM complications, including vascular damage [10].

Cortisol, a glucocorticoid hormone, has emerged as a significant factor in T2DM pathogenesis. Cortisol dysregulation, particularly hypercortisolism, is associated with insulin resistance, β-cell dysfunction, and metabolic disturbances, which are common in T2DM. Elevated cortisol levels are positively correlated with glycated hemo-globin (HbA1c) levels and are more prevalent among T2DM patients than in the general population [11]. A sys-tematic review involving 2,827 T2DM patients reported a 3.4% prevalence of hypercortisolism, which is linked to more severe complications such as hypertension and arterial stiffness [12].

Emerging evidence suggests a potential interplay between miRNA-146-5p and cortisol in T2DM pathogenesis. miRNA-146-5p regulates inflammatory pathways that intersect with the effects of cortisol immune and metabolic responses. Dysregulation of either pathway can disrupt homeostasis and promote chronic inflammation and metabolic disturbances [13]. Additionally, the hypothalamic-pituitary-adrenal (HPA) axis, a central regulator of cortisol secretion, may influence miRNA expression and activity. However, the mechanisms linking miRNA-146-5p and cortisol remain poorly understood, presenting a critical gap in the current knowledge [14]. Investigating this interplay could provide new insights into T2DM pathophysiology and aid in identifying novel therapeutic targets.

The gap in understanding regarding the connection between cortisol, miRNA-146-5p, and T2DM pathogenesis lies in the lack of comprehensive knowledge about how these factors interact to drive disease progression. While both cortisol dysregulation and miRNA-146-5p have been separately implicated in T2DM, their combined role in modulating inflammation, metabolic disturbances, and vascular complications remains poorly understood. Specifically, the mechanisms by which cortisol influences miRNA-146-5p expression, and how this interaction contributes to chronic inflammation and β-cell dysfunction, have not been sufficiently explored. Additionally, the potential feedback loop between cortisol and miRNA-146-5p in the context of T2DM pathophysiology, especially through the hypothalamic-pituitary-adrenal (HPA) axis, has not been well investigated. This gap highlights the need for a more integrated approach to understanding the molecular and hormonal pathways that underpin T2DM progression.

Therefore, this study aimed to investigate the diagnostic and pathophysiological relevance of miRNA-146 and cortisol in Type 2 Diabetes Mellitus (T2DM). Specifically, we evaluated their roles as potential biomarkers for identifying metabolic and inflammatory abnormalities that are characteristic of T2DM. Additionally, this study explored the relationship between miRNA-146, cortisol, and glycemic markers such as HbA1c and RBG, to un-cover their interplay in driving chronic inflammation and glucose dysregulation. By establishing cutoff values and diagnostic performance metrics, this study sought to enhance early detection and provide insights into novel therapeutic targets for managing T2DM progression.

2. Experimental (subject and methods)

2.1. Study Population

This cross-sectional study included 200 participants divided into two groups: Group 1 (T2DM patients): 100 participants with type 2 Diabetes Mellitus (T2DM) and Group 2 (Healthy controls): 100 participants without diabetes as controls.

A sample size of 200 participants (100 T2DM patients and 100 healthy controls) is justified based on the need for statistical power to detect significant differences between groups, the ability to perform reliable subgroup analyses, and the goal of providing precise, and valid findings. Previous studies with comparable aims may have used sample sizes in the range of 100–150 participants per group [15, 16] which supports the justification for 200 participants in this study as an adequate and typical sample size for similar research. Accordingly, this sample size ensures that the study has sufficient power to detect moderate to large effect sizes, control for confounding variables (e.g., age, sex, comorbid conditions), and achieve accurate and meaningful conclusions related to T2DM and its associated biomarkers.

Data collection and sample acquisition were conducted at the outpatient clinic of the Faculty of Medicine at Suez Canal University.

Inclusion Criteria: Participants with T2DM were selected based on diagnosis of impaired glucose tolerance or fasting insulin levels, or a confirmed diagnosis of T2DM. Participants were diagnosed with T2DM based on specific biochemical parameters. These included impaired glucose tolerance and elevated fasting insulin levels, with a cut-off value of 7.45. This is supported by findings showing that fasting insulin levels higher than this threshold were significantly associated with T2DM.

Exclusion Criteria: Participants were excluded from the study if they had any of the following conditions: adrenal diseases such as Cushing syndrome, pheochromocytoma, or primary hyperaldosteronism; a history of central nervous system (CNS) surgery or pituitary gland disorders; acute or chronic infections or stress conditions, including myocardial infarction or diabetic ketoacidosis; recent administration of steroids within the preceding three months; liver failure or cirrhosis; a history of malignancies or severe hypoglycemia within the past three months; or severe electrolyte disturbances. The study excluded also patients with common T2DM-related complications, such as hypertension, nephropathy, neuropathy, or retinopathy. The inclusion of such patients could introduce variability in the findings, as these complications are often associated with changes in inflammatory and metabolic profiles, including cortisol and miRNA-146-5p levels.

The exclusion criteria were designed to ensure that the study focuses specifically on the metabolic and bio-chemical characteristics of individuals with T2DM, without interference from other diseases, treatments, or conditions that could confound the results. By excluding individuals with conditions that influence hormonal regulation, glucose metabolism, and electrolyte balance, the study can more accurately isolate and investigate the pathophysiological mechanisms underlying T2DM. This approach enhances the internal validity of the study and ensures that any observed associations between biomarkers and T2DM are not influenced by external factors.

2.2. Overnight Dexamethasone Suppression Test (DST)

An overnight dexamethasone suppression test was performed to rule out preclinical Cushing's syn-drome. The participants received 1 mg of dexamethasone orally at 11:00 p.m., followed by the measurement of serum cortisol at 8:00 a.m. the next morning. A cortisol level below 50 nmol/L was considered sufficient suppression, ruling out preclinical Cushing's syndrome.

2.3. Clinical Significance

Overnight DST is critical for detecting subtle hypercortisolism, which can mimic or exacerbate metabolic abnormalities such as insulin resistance and hyperglycemia in T2DM. Identifying and excluding preclinical Cushing's syn-drome ensures that the observed metabolic and molecular findings are directly attributable to T2DM rather than con-founding endocrine conditions.

2.4. Biochemical Parameter Measurement and Data Collection

Biochemical assessments were conducted for all participants, including patients with T2DM and healthy controls. These evaluations included glucose metabolism, lipid profile, vitamin D levels, and serum cortisol levels, providing a comprehensive view of metabolic health. The specific tests and procedures are as follows:

- Glucose and Lipid Profile: Random blood glucose (RBG), fasting insulin, HbA1c, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured. The LDL-C levels were calculated using the Friedewald formula [17];

LDL-c= total cholesterol - (HDL-c + triglycerides/5). This formula aids in estimating LDL-C concentration when direct measurement is not feasible, thereby offering insights into cardiovascular risk factors.

- Vitamin D Levels: Quantitative assessment of 25-hydroxy vitamin D in the serum was performed using a highly sensitive enzyme-linked immunosorbent assay (ELISA) kit (IBL International GmbH, Hamburg, Germany; RE53041). The vitamin D status is crucial for evaluating its potential impact on glucose metabolism and insulin sensitivity.
- Cortisol Levels: Serum cortisol, a key hormone implicated in stress and metabolic regulation, was quantified at 8:00 a.m. using a competitive ELISA kit (Monobind, Lake Forest, CA, USA; Cat# 3625-300A). Cortisol levels reflect the peak of the diurnal rhythm and are instrumental in identifying dysregulation potentially linked to T2DM pathogenesis.

To obtain these parameters, a 10 mL venous blood sample was collected from each participant by trained laboratory personnel. The blood was processed as follows:

- RNA Extraction: Five milliliters of the sample was placed in EDTA tubes for RNA extraction, ensuring the preservation of nucleic acids critical for subsequent miRNA analysis.
- Biochemical Analysis: The remaining five milliliters was allowed to clot at room temperature. The serum was separated by centrifugation and analyzed using an Olympus automatic biochemistry analyzer AU400. The analyzer utilized original reagents from Olympus Diagnostics to ensure the precision and reliability of the measurements.

These carefully executed procedures not only ensured the accuracy of the biochemical data but also provided a robust foundation for evaluating the interplay between biochemical markers and T2DM pathogenesis.

2.5. Determination of Serum miRNA-146 Levels

The serum levels of miRNA-146 were analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) to ensure precise quantification of this microRNA. The procedure involved the following steps.

- RNA Extraction and Purification: Total RNA was extracted from serum samples using the miRNeasy Mini Kit (Qi-agen, Germany; Cat. # 217004) according to the manufacturer's protocol to ensure high-quality RNA for downstream applications.
- cDNA Synthesis: Reverse transcription was performed to synthesize complementary DNA (cDNA) using the Taq-Man miRNA Reverse Transcription Kit (Applied Biosystems; Catalogue # 4366596). This step is crucial for converting RNA into a stable format suitable for amplification and quantification.
- qPCR Analysis: Quantitative PCR (qPCR) was conducted using the TaqMan Universal Master Mix and a specific miRNA-146 assay (Cat. # 4427975, ID: 002623). To ensure normalization and reliability, RNU49 (Cat. # PN4427975, ID: 001005) served as the housekeeping gene, allowing for the accurate relative quantification of miRNA-146 levels.

Expression Quantification: The expression levels of miRNA-146 were calculated using the 2^{ΔΔCt} method, a widely recognized approach for relative quantification in All qPCR analyses were performed using a 5-plex Rotor-Gene PCR Analyzer (Qiagen, Germany), which offers high sensitivity and precision and is essential for detecting subtle variations in miRNA expression. These results provide key insights into the role of miRNA-146 in the pathophysiology of T2DM and its potential as a biomarker.

2.6. Statistical Analysis

Data analysis was performed using SPSS version 22 (IBM Corp., Armonk, NY, USA). Numerical data are presented as mean ± standard deviation (SD) or median (interquartile range), while categorical data are expressed as numbers and percentages. Student's t-test was used for comparisons of parametric variables, whereas the Mann-Whitney U test was used for non-parametric variables. The chi-squared test was used to analyze categorical data. The relationships between variables were assessed using Spearman's correlation coefficient. Receiver Operating Characteristic (ROC) curves were generated to determine the cut-off values for fasting insulin, serum cortisol, and miRNA-146 levels. A P-value <0.0001 was considered statistically significant.

Data statement All information is accessible from the corresponding author upon request

3. Results and Discussion

3.1. Demographic Clinical and Biochemical Characteristics

The demographic, clinical, and biochemical characteristics of the study participants are detailed in Tables 1 and 2, encompassing two groups: Type 2 Diabetes Mellitus (T2DM) patients and healthy controls. T2DM patients exhibited significantly higher levels of Random Blood Glucose (RBG), fasting insulin, and HbA1c than controls ($p<0.05$). Lipid profiles revealed elevated total cholesterol, triglyceride, LDL levels, and reduced HDL levels ($p<0.05$). Additionally, serum vitamin D and miRNA-146 levels were significantly lower in T2DM patients, with miRNA-146 levels at 4 ± 7.5 versus 21.8 ± 20.9 in controls ($p<0.05$).

Table 3 shows the correlation of cortisol, fasting insulin, Vitamin D & miRNA- 146 with other variables in T2DM cases. Corti-sol levels were positively associated with random blood glucose (RBG) ($r = 0.636$, $p = 0.045$) and HbA1c ($r = 0.467$, $p = 0.044$), highlighting the role of cortisol in both acute and chronic glycemic dysregulation in T2DM patients. However, cortisol levels showed no significant correlation with lipid profile parameters. Fasting insulin levels did not demonstrate meaningful associations with miRNA-146, vitamin D, or lipid variables. Similarly, miRNA-146 and vitamin D levels were not significantly correlated with the cortisol, insulin, or lipid profiles. Lipid parameters (cholesterol, TG, HDL, and LDL) also lacked significant interrelations with these biomarkers.

Table 4 shows the correlation of Cortisol, fasting insulin, Vitamin D & miRNA- 146 with other variables with-in the healthy controls

In healthy individuals, cortisol levels did not significantly correlate with other variables, including fasting insulin, vitamin D, miRNA-146, or metabolic parameters, such as RBG, cholesterol, TG, HDL, HbA1c, and LDL. Fasting insulin showed a weak, non-significant positive correlation with vitamin D levels ($r = 0.130$, $p = 0.197$), suggesting a minor potential association. miRNA-146 levels exhibited a significant negative correlation with HDL ($r = -0.229$, $p = 0.022$), implying that higher miRNA-146 expression may be linked to lower HDL levels. However, no significant correlations were found with other variables such as cortisol, fasting insulin, age, RBG, or lipid profiles

3.2. Cutoff Values for Biomarkers

Receiver Operating Characteristic (ROC) curves were used to determine the cut-off values for key biomarkers associated with T2DM (Table 5 and Figure 2).

Cortisol as a Biomarker: Cortisol demonstrated strong diagnostic potential for distinguishing T2DM patients from healthy controls, with a cut-off value of ≥ 3.8 nmol/L. The sensitivity was 95.0% and the specificity was 71.0%, with an Area Under the Curve (AUC) of 0.819 (95% CI: 0.759–0.878, $p = 0.0001$). These results indicated excellent sensitivity, making cortisol a reliable marker for identifying individuals with T2DM. However, its relatively lower specificity suggests some overlap with non-diabetic individuals, likely because of factors such as stress or other metabolic conditions that can elevate cortisol levels.

Vitamin D as a Biomarker: Vitamin D levels had a cut-off value of ≥ 32.5 ng/mL, with a sensitivity of 75.0% and specificity of 59.0%. The AUC was 0.700 (95% CI: 0.625–0.775, $p = 0.0001$). These findings suggest moderate diagnostic utility, with vita-min D exhibiting acceptable sensitivity, but low specificity. This indicates that while vitamin D levels may provide insights into metabolic dysregulation, they are less robust as standalone indicators of T2DM because of widespread vitamin D deficiency in the general population.

miRNA-146 as a Biomarker: miRNA-146 emerged as the most effective biomarker among the three, with a cut-off value of ≤ 6.83, sensitivity of 83.0%, and specificity of 79.0%. The AUC was the highest at 0.832 (95% CI: 0.772–0.893, $p = 0.0001$). This indicates that miRNA-146 has strong diagnostic accuracy, offering both high sensitivity and specificity. Its involvement in the inflammatory and metabolic pathways further highlights its potential as a reliable and disease-specific biomarker for T2DM diagnosis.

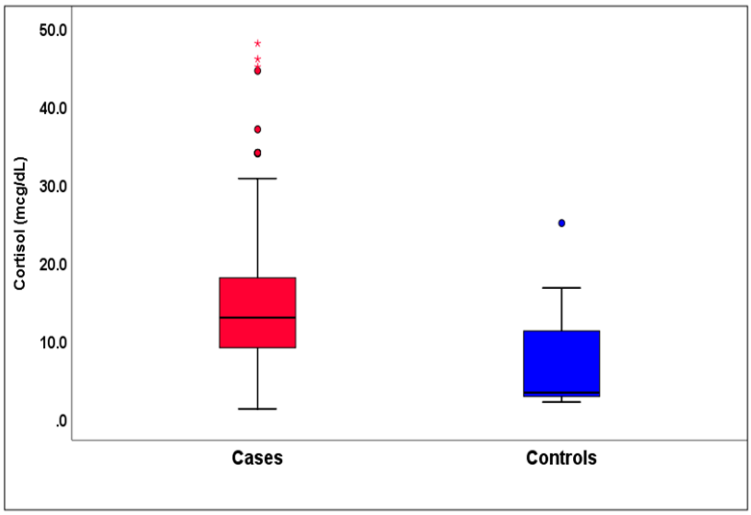
Fasting Insulin: A fasting insulin value greater than 7.45 was associated with T2DM, yielding an AUC of 0.819, 80.0% sensitivity, and 73.0% specificity.

Table 1: Demographics of T2DM cases and healthy subjects

	T2DM cases (n=100) N (%)	**Controls (n=100) N (%)**
Gender		
Male	35 (35)	53 (53)
Female	65 (65)	47 (47)
Age (years)		
Range	17 – 50	20 – 67
Mean ± SD	26.7 ± 6.1	41.7 ± 11.2
Median (IQR)	25 (22 - 31)	40 (34.5 - 46.5)

Table 2: Biochemical parameters of T2DM patients and healthy subjects.

	T2DM Cases (n=100)	**Controls (n=100)**	**p-value**
RBG (million/mm3)			
Mean ± SD	256 ± 101.2	95.1 ± 10.7	**0.0001**
Median (IQR)	255 (177.5 - 318.5)	96.5 (89 - 100)	
Fasting insulin (mIU/L)			
Mean ± SD	10.4 ± 3.7	6.3 ± 2.6	**0.0001**
Median (IQR)	11 (8 - 13)	6 (4.1 - 8)	
HbA1c (mg/dl)			
Mean ± SD	6.2 ± 2.1	3.7 ± 0.5	**0.0001**
Median (IQR)	6.6 (4 - 7.9)	3.8 (3.6 - 4)	
Cholesterol (mg/dl)			
Mean ± SD	172.9 ± 41	148.4 ± 18.6	**0.0001**
Median (IQR)	170 (142 - 200)	147 (134 - 156)	
TG (mg/dl)			
Mean ± SD	189.8 ± 24.8	146.5 ± 23	**0.0001**
Median (IQR)	189 (180 - 190)	144 (125 - 156)	
HDL (mg/dl)			
Mean ± SD	32.9 ± 9.9	43.9 ± 8.6	**0.0001**
Median (IQR)	30 (25 - 38)	41 (40 - 46)	
LDL (mg/dl)			
Mean ± SD	127.1 ± 27.7	107.7 ± 11	**0.0001**
Median (IQR)	121.5 (102 - 152)	103 (99 - 112.5)	
Cortisol (nmol/L)			
Mean ± SD	15.6 ± 10.2	6.3 ± 5.5	**0.0001**
Median (IQR)	12.9 (9.1 - 18)	3.3 (2.8 - 11.2)	
Vitamin D (ng/mL)			
Mean ± SD	33.2 ± 20	41.1 ± 16.9	**0.0001**
Median (IQR)	38 (31.5 - 45)	30 (20 - 38.5)	
MiRNA-146			
Mean ± SD	4 ± 7.5	21.8 ± 20.9	**0.0001**
Median (IQR)	2 (1.1 - 4)	16 (8 - 32)	



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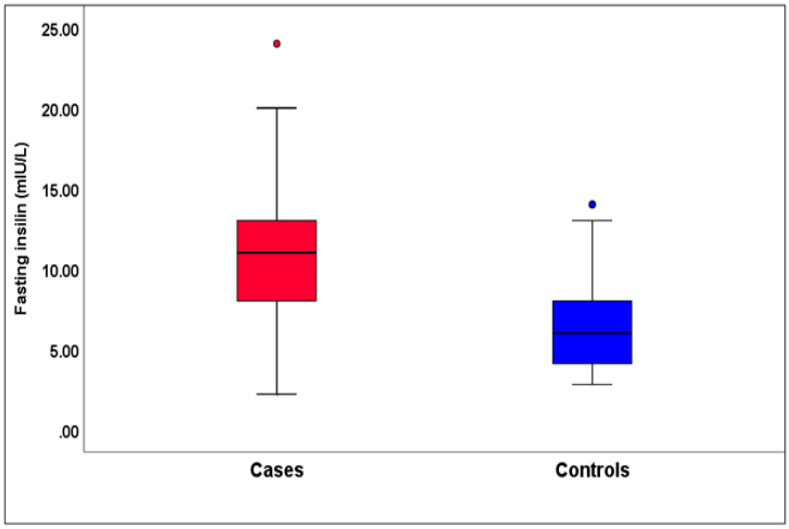

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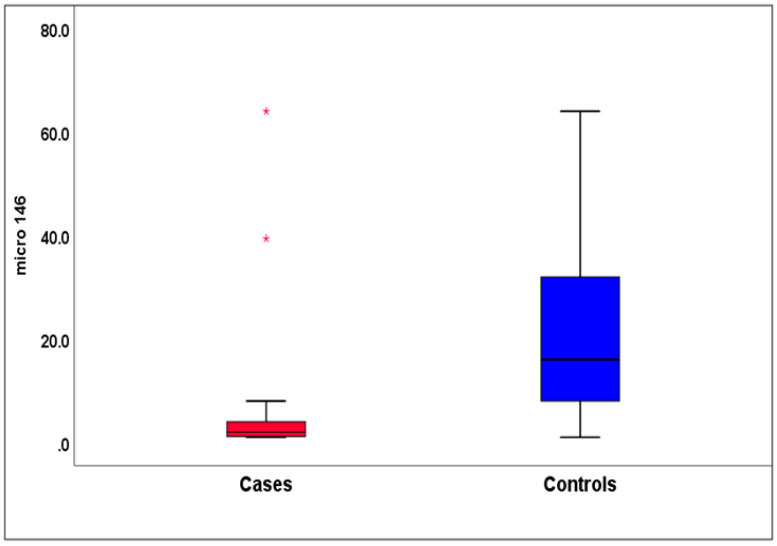

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Figure 1: A) Serum level of cortisol in T2DM subjects and healthy subjects, B) Serum level of fasting insulin in T2DM subjects and healthy subjects, C) Expression level of miRNA-146 in T2DM subjects and healthy subjects.

Table 3: Correlation of Cortisol, fasting insulin, Vitamin D & miRNA- 146 with other variables within T2DM cases

Parameter		Cortisol	Fasting insulin	MiRNA-146
Fasting insulin (mIU/L)	**r**	-0.140		
	P	0.164		
Vitamin D (ng/ML)	**r**	-0.049	-0.004	
	P	0.626	0.965	
MiRNA-146	**r**	-0.058	-0.064	
	P	0.565	0.528	
Duration of DM (years)	**r**	-0.028	0.169	-0.118
	P	0.780	0.092	0.242
Age	**r**	-0.131	0.090	0.016
	P	0.194	0.372	0.875
RBG (million/mm3)	**r**	0.636**	-0.105	0.088
	P	0.045	0.300	0.385
Cholesterol (mg/dl)	**r**	0.090	-0.181	-0.029
	P	0.371	0.071	0.773
TG (mg/dl)	**r**	0.143	-0.166	-0.106
	P	0.157	0.099	0.296
HDL (mg/dl)	**r**	-0.029	0.012	0.024
	P	0.772	0.902	0.813
HbA1c %	**r**	0.467**	-0.046	0.016
	P	0.044	0.652	0.874
LDL (mg/dl)	**r**	0.162	-0.095	0.070
	P	0.107	0.349	0.487

Table 4: Correlation of Cortisol, Fasting insulin, Vitamin D & miRNA-146 with other variables within healthy subjects

parameters		Cortisol	Fasting insulin	MiRNA-146
Fasting insulin (mIU/L)	**R**	0.086		
	P	0.395		
Vitamin D (ng/mL)	**R**	0.067	0.130	
	P	0.507	0.197	
MiRNA-146	**R**	0.089	0.056	
	P	0.380	0.577	
Age	**R**	-0.091	-0.043	-0.142
	P	0.368	0.673	0.158
RBG (million/mm3)	**R**	-0.072	-0.047	-0.015
	P	0.479	0.641	0.885
Cholesterol (mg/dl)	**R**	-0.079	0.029	0.002
	P	0.435	0.774	0.984
TG (mg/dl)	**R**	-0.014	0.098	0.036
	P	0.892	0.330	0.723
HDL (mg/dl)	R	0.024	0.183	-.229*
	P	0.809	0.068	0.022
HbA1c (mg/dl)	R	-0.024	-0.044	-0.161
	P	0.812	0.665	0.109
LDL (mg/dl)	R	-0.115	0.000	-0.040
	P	0.255	0.999	0.694

Table 5: Evaluation of AUC and optimal cut-off values of Cortisol, Vitamin D, and MiRNA-146

	Cut-off	Sensitivity	Specificity	AUC	95%CI			P value
Cortisol (nmol/L)	≥ 3.8	95.0%	71.0%	0.819	0.759	-	0.878	0.0001
Vitamin D (ng/mL)	≥ 32.5	75.0%	59.0%	0.700	0.625	-	0.775	0.0001
MiRNA-146	≤ 6.83	83.0%	79.0%	0.832	0.772	-	0.893	0.0001

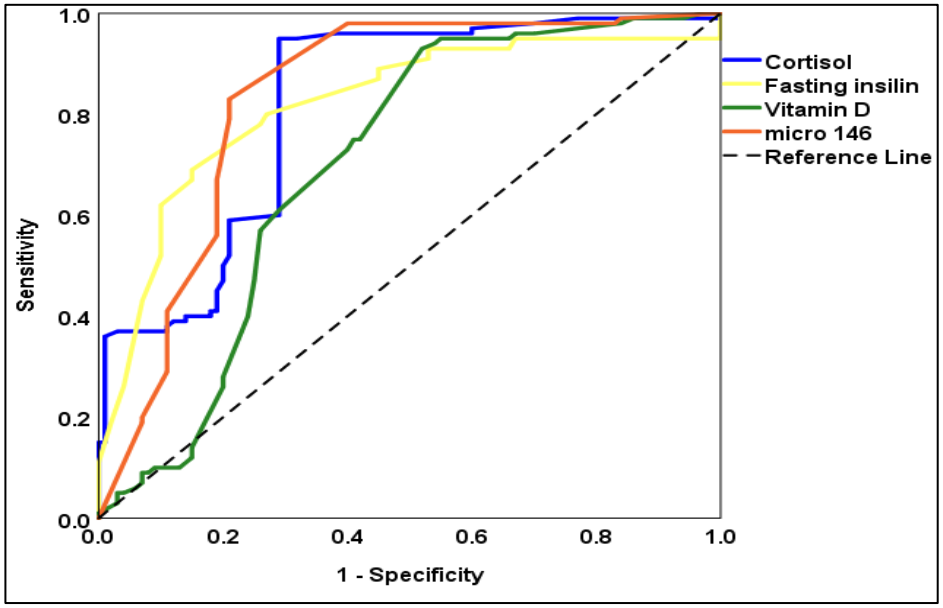

Figure 2: Receiver operating characteristic curve for Cortisol, Fasting insulin, Vitamin D, and MiRNA-146. (AUC= 0.819, 0.819, 0.700, and 0.832 respectively, p= 0.0001).

4. Discussion

4.1. Interplay Between miRNA-146-5p and Cortisol in T2DM Pathogenesis

This study addresses a significant gap in the understanding of type 2 Diabetes Mellitus (T2DM) by exploring the interplay between miRNA-146-5p and cortisol. While miRNAs, such as miRNA-146-5p regulate inflammatory and metabolic pathways, their exact roles in T2DM, particularly in conjunction with cortisol, have not been well elucidated. This study fills this gap by examining how miRNA-146-5p influences inflammation and interacts with cortisol in the context of T2DM, offering a more integrated view of the pathophysiology of the disease.

4.2. Role of miRNA-146-5p in the Pathogenesis of T2DM and Regulating Inflammation and Metabolism

In this study, we found that miRNA-146-5p expression was significantly lower in T2DM patients compared to healthy controls. This aligns with growing evidence that miRNAs, including miRNA-146-5p, play a crucial role in regulating glucose and lipid metabolism, insulin production, and pancreatic β-cell differentiation [18]. miRNA-146-5p has been implicated in modulating immune responses by interacting with the Toll-like receptor (TLR) signalling pathway, directly influencing Interleukin-1 Receptor-Associated Kinase (IRAK1) and Tumor Necrosis Factor Receptor-Associated Factor 6 (TRAF6) [19, 20]. By downregulating these targets, miRNA-146-5p limits Nuclear Factor Kappa B (NF-κB) activation, which suppresses the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [21,22]. Dysregulation of miRNA-146-5p, as observed in T2DM, leads to excessive activation of these inflammatory pathways, contributing to chronic inflammation, insulin resistance, and β-cell dysfunction—hallmarks of T2DM.

In addition to its anti-inflammatory role, miRNA-146-5p influences metabolic processes by regulating insulin signaling and β-cell function. Reduced miRNA-146-5p expression correlates with increased production of inflammatory cytokines and impaired insulin receptor signaling, exacerbating insulin resistance [23]. Hyperglycemia-induced activation of NF-κB further amplifies this inflammatory response, promoting cytokine release and the progression of T2DM-related complications, such as vascular damage [24, 25]. Moreover, miRNA-146-5p indirectly regulates glucose uptake by inhibiting stress kinases like JNK and p38 MAPK, which are linked to insulin resistance in adipose tissue and skeletal muscle [26, 27]. The multifaceted role of miRNA-146-5p in T2DM pathogenesis highlights its importance as both a regulator of immune and metabolic pathways and a potential therapeutic target for mitigating inflammation, improving insulin sensitivity, and preserving β-cell function.

4.3. Cortisol Dysregulation in T2DM

Cortisol dysregulation has long been recognized as a key factor in T2DM, where elevated cortisol levels are associated with insulin resistance and β-cell dysfunction [26]. This study found significantly higher cortisol levels in T2DM patients than in healthy controls. These findings support previous research linking hypercortisolism with impaired insulin signaling, glucose metabolism, and insulin resistance characteristics of T2DM [28-30]. Prolonged exposure to glucocorticoids suppresses insulin secretion, reduces glucose metabolism in β-cells, and impairs the function of the glucose transporter GLUT-2, contributing to glucose intolerance [31-34]. Our results also highlight a positive correlation between cortisol levels and glycemic markers, including HbA1c and random blood glucose (RBG), which highlights the role of cortisol in exacerbating glucose dysregulation. Elevated cortisol promotes hepatic gluconeogenesis, reduces peripheral glucose uptake, and impairs insulin sensitivity, contributing to hyperglycemia and poor glycemic control in individuals with type 2 diabetes mellitus (T2DM). Studies have demonstrated that increased cortisol exposure is associated with worsened glycemia in T2DM patients [35). Additionally, elevated fasting plasma cortisol levels have been linked to features of the metabolic syndrome and a higher prevalence of ischemic heart disease among T2DM patients [36]. Furthermore, research indicates that stress-induced cortisol release can impair insulin sensitivity, leading to elevated blood glucose levels [37].

4.4. The Role of Vitamin D in the Pathogenesis of T2DM:

Vitamin D plays a crucial role in glucose metabolism and insulin sensitivity, making its deficiency a significant factor in the pathogenesis of Type 2 Diabetes Mellitus (T2DM). Vitamin D influences β-cell function in the pancreas by binding to the vitamin D receptor (VDR), which is expressed in pancreatic islets. This interaction enhances insulin secretion by regulating calcium homeostasis within β-cells, a process essential for insulin exocytosis. Additionally, vitamin D modulates insulin sensitivity by regulating the expression of insulin receptor genes in peripheral tissues and reducing systemic inflammation, which contributes to insulin resistance. Studies have demonstrated an inverse relationship between serum 25-hydroxyvitamin D levels and the risk of developing T2DM. For instance, individuals with low vitamin D levels are at a higher risk of glucose intolerance, impaired insulin secretion, and inflammation, which are hallmarks of T2DM [38, 39]. Furthermore, vitamin D inhibits the activation of pro-inflammatory cytokines, such as TNF-α and IL-6, through its effect on nuclear factor-kappa B (NF-κB) pathways, reducing chronic inflammation associated with T2DM pathogenesis [40]. Despite these insights, the precise mechanisms by which vitamin D deficiency exacerbates T2DM require further investigation to establish causal relationships and the potential benefits of supplementation as a preventive or therapeutic strategy.

4.5. Integrating miRNA-146-5p and Cortisol as Complementary Biomarkers

The hypothalamic-pituitary-adrenal (HPA) axis is a central component in the regulation of cortisol, and our findings suggest that cortisol dysregulation may influence miRNA-146-5p expression. The feedback loop between these two molecules highlights a critical interaction between endocrine and molecular pathways in T2DM [28]. miRNA-146-5p's regulatory effects on inflammation and its potential interaction with cortisol can help explain how chronic inflammation contributes to the development and progression of T2DM. By integrating miRNA-146-5p and cortisol as complementary biomarkers, this study provides a more comprehensive model to understanding T2DM's complex pathophysiology.

The interplay between miRNA-146-5p and cortisol has direct implications for glucose metabolism. Elevated cortisol levels impair glucose uptake by muscle and adipose tissues, mainly by inhibiting the translocation of GLUT4, a key glucose transporter [41, 42]. Cortisol also promotes gluconeogenesis and lipolysis in the liver and lipolysis, both of which contribute to elevated blood glucose levels. This cortisol-driven dysregulation is likely exacerbated in T2DM, where elevated levels of circulating free fatty acids (FFA) and ceramides further disrupt insulin signaling [43].

In T2DM, miRNA-146-5p appears to be a crucial regulator of NF-κB, which in turn modulates insulin signaling and glucose homeostasis. Studies have shown that hyperglycemia reduces miRNA-146-5p levels, thereby enhancing the inflammatory response through NF-κB activation. This, in turn, may further increase cortisol levels and amplify insulin resistance [44]. crosstalk between miRNA-146-5p and cortisol highlights the complex feedback loop that underpins the metabolic disturbances and inflammation observed in T2DM.

4.6. Therapeutic Implications and Future Directions

This study has significant therapeutic implications, suggesting that restoring normal miRNA-146-5p expression could mitigate the inflammation and metabolic dysfunction observed in T2DM. Moreover, cortisol could

serve as a secondary biomarker for assessing disease progression and identifying individuals at a high risk of diabetic complications. Future research should explore potential interventions that aim to normalize miRNA expression or target cortisol dysregulation, thereby offering new avenues for preventive and therapeutic strategies in T2DM management [45-47]. Additionally, combination therapies involving miRNA-146-5p mimics or anti-miRNA oligonucleotides could be beneficial in treating T2DM-related complications and improving insulin sensitivity and glucose control [48].

This study contributes significantly to the growing body of knowledge on the roles of miRNA-146-5p and cortisol in T2DM pathogenesis. By shedding light on their interconnected roles, this study opens up possibilities for more effective diagnostic and therapeutic strategies. The integration of these biomarkers into clinical practice could lead to more precise monitoring and early detection of T2DM, ultimately improving patient outcome.

This study demonstrates several notable strengths, primarily its focus on novel biomarkers, miRNA-146 and cortisol, which are increasingly recognized for their pivotal roles in metabolic and inflammatory pathways in Type 2 Diabetes Mellitus (T2DM). By assessing their diagnostic utility and mechanistic implications, the study provides valuable insights into the pathophysiology of T2DM. A comprehensive diagnostic approach was employed, including the establishment of cutoff values for sensitivity, specificity, and Area Under the Curve (AUC), enhancing the clinical relevance of these biomarkers as tools for early T2DM detection. The study's use of Receiver Operating Characteristic (ROC) curves facilitated precise evaluations of biomarker effectiveness in distinguishing T2DM patients from healthy controls, strengthening the potential for clinical applications. Correlation analyses further enriched the findings by examining the relationships between cortisol, miRNA-146, and glycemic markers (e.g., HbA1c and RBG), offering insights into the interaction of these biomarkers within the context of chronic inflammation and glucose dysregulation. Additionally, robust methodologies, including RT-qPCR for miRNA-146 analysis and ELISA for cortisol and vitamin D, ensured high precision and reliability of biomarker quantification. The inclusion of a wide range of biochemical parameters, such as lipid profiles, glucose levels, and vitamin D status, provided a comprehensive understanding of metabolic health in T2DM, adding depth to the study's findings.

Despite the study strengths, the study has several limitations. First, while the sample size was adequate, expanding it could improve the statistical power and allow for subgroup analyses, particularly for variations in disease severity or treatment history. Additionally, the lack of strict demographic or clinical matching between the T2DM patients and healthy controls (e.g., age, sex, and BMI) may introduce confounding factors, potentially biasing the comparison. Another notable limitation is that the study did not measure body mass index (BMI) or central obesity markers (waist and hip circumferences) and their potential impact on fasting insulin levels and lipid profiles. Given the strong association between obesity and T2DM, this omission may limit a comprehensive understanding of how these factors influence metabolic health in the context of T2DM. Furthermore, the study excluded patients with common T2DM-related complications, such as hypertension, nephropathy, neuropathy, or retinopathy. Accordingly, the study's findings may be less applicable to the broader T2DM population, where such complications are prevalent. Lastly, the limited exploration of mechanistic pathways and the lack of population diversity constrain the generalizability of the findings. Future studies should incorporate longitudinal designs, larger and more diverse populations, and a deeper investigation into the molecular mechanisms linking miRNA-146-5p and cortisol to T2DM pathophysiology.

5. Conclusions

This study highlights miRNA-146 and cortisol as promising biomarkers for Type 2 Diabetes Mellitus (T2DM). miRNA-146 shows strong diagnostic accuracy with high sensitivity and specificity, making it a valuable tool for early detection, while cortisol, despite its sensitivity, requires confirmatory testing due to some overlap with non-T2DM individuals. Vitamin D was less effective as a diagnostic marker, reflecting its broader role in metabolic health.

The findings emphasize the link between cortisol dysregulation and glycemic markers like HbA1c and RBS, implicating cortisol and miRNA-146-5p in T2DM pathophysiology. Dysregulation of these biomarkers contributes to inflammation, insulin resistance, and β-cell dysfunction. miRNA-146-5p emerges as a novel therapeutic target for modulating immune and metabolic dysfunction, with its interaction with cortisol in the HPA axis offering potential for targeted interventions.

Integrating miRNA-146 and cortisol as complementary biomarkers could enhance T2DM diagnosis and monitoring. Further research is needed to validate these findings, optimize clinical applications, and develop therapies targeting these biomarkers to improve T2DM management.

6. Recommendations for Future Research

To build upon these findings, future studies should consider increasing the sample size and improving control group matching to enhance statistical power and reduce confounding. Longitudinal studies would provide insights into the causal relationship between cortisol, miRNA-146, and T2DM, offering a clearer understanding of their roles in disease progression. Additionally, exploring mechanistic pathways could provide more in-depth insights into how these biomarkers contribute to T2DM's metabolic and inflammatory processes. Expanding this study to include more diverse populations would also enhance the generalizability of the findings, ensuring that the identified biomarkers are relevant across different demographic groups.

7. Conflicts of interest

Declaration: Competing interest Authors attest to having no conflicting interests.

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All author contributes equally in the paper

10. References

[1] Zeinali F, Zarch SMA, Jahan-Mihan A, et al. Circulating microRNA-122, microRNA-126-3p, and microRNA-146a are associated with inflammation in patients with pre-diabetes and type 2 diabetes mellitus: A case control study. PLoS One. 2021; 16(66): 1-13. doi:10.1371/journal.pone.0251697

[2] Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of Type 2 Diabetes Mellitus. International journal of molecular sciences, 21(17), 6275. doi.org/10.3390/ijms21176275

[3] Dunlay SM, Givertz MM, Aguilar D, et al. Type 2 Diabetes Mellitus and Heart Failure: A Scientific Statement From the American Heart Association and the Heart Failure Society of America: This statement does not represent an update of the 2017 ACC/AHA/HFSA heart failure guideline update. Circulation. 2019; 140(7): E294-E324. doi:10.1161/CIR.0000000000000691

[4] Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019; 157: 107843. doi:10.1016/j.diabres.2019.107843

[5] Krentz NAJ, Gloyn AL. Insights into pancreatic islet cell dysfunction from type 2 diabetes mellitus genetics. Nat Rev Endocrinol 2020 164. 2020; 16(4): 202-212. doi:10.1038/s41574-020-0325-0

[6] Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008; 18(10): 997-1006. doi:10.1038/cr.2008.282

[7] Chen H, Lan HY, Roukos DH, Cho WC. Application of microRNAs in diabetes mellitus. J Endocrinol. 2014; 222(1). doi:10.1530/JOE-13-0544

[8] Smit-McBride Z, Morse LS. MicroRNA and diabetic retinopathy—biomarkers and novel therapeutics. Ann Transl Med. 2021; 9(15): 1280-1280. doi:10.21037/atm-20-5189

[9] Matsha TE, Kengne AP, Hector S, Mbu DL, Yako YY, Erasmus RT. MicroRNA profiling and their pathways in South African individuals with prediabetes and newly diagnosed type 2 diabetes mellitus. Oncotarget. 2018; 9(55): 30485-30498. doi:10.18632/oncotarget.25271

[10] Wang J, Chen J, Sen S. MicroRNA as Biomarkers and Diagnostics. J Cell Physiol. 2016; 231(1): 25-30. doi:10.1002/JCP.25056

[11] Chatterjee B, Sarkar M, Bose S, et al. MicroRNAs: Key modulators of inflammation-associated diseases. Semin Cell Dev Biol. 2024 15; 154(Pt C): 3 64-373.2023; . doi:10.1016/j.semcdb.2023.01.009

[12] Steffensen C, Pereira A.M, Dekkers O and Jørgensen J.O. DIAGNOSIS OF ENDOCRINE DISEASE: Prevalence of hypercortisolism in type 2 diabetes patients: a systematic review and meta-analysis. European journal of endocrinology. 2016; 175(6), R247–R253. doi.org/10.1530/EJE-16-0434

[13]- Guo H, Wu H, Li Z. The Pathogenesis of Diabetes. Int J Mol Sci. 2023 Apr 10;24(8):6978. doi: 10.3390/ijms24086978. PMID: 37108143; PMCID: PMC10139109.

[14] Kluwe B, Ortiz R, Odei JB, et al. The Association of Cortisol Curve Features with Incident Diabetes among Whites and African Americans: The CARDIA Study. Psychoneuroendocrinology. 2021; 123: 105041. doi:10.1016/J.PSYNEUEN.2020.105041

[15] Pordzik, J., Jakubik, D., Jarosz-Popek, J. et al. Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: bioinformatic analysis and review. Cardiovasc Diabetol 18, 113 (2019). https://doi.org/10.1186/s12933-019-0918-x

[16] Long, J., Yang, Z., Wang, L. et al. Metabolite biomarkers of type 2 diabetes mellitus and pre-diabetes: a systematic review and meta-analysis. BMC Endocr Disord 20, 174 (2020). https://doi.org/10.1186/s12902-020-00653-x

[17] Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, 18(6), 499–502. https://doi.org/10.1093/clinchem/18.6.499

[18] Aladel A, Khatoon F, Khan MI, et al. Evaluation of miRNA-143 and miRNA-145 Expression and Their Association with Vitamin-D Status Among Obese and Non-Obese Type-2 Diabetic Patients. J Multidiscip Healthc. 2022; 15: 2979-2990. doi:10.2147/jmdh.s391996

[19] Abdel Mageed SS, Doghish AS, Ismail A, et al. The role of miRNAs in insulin resistance and diabetic macrovascular complications – A review. Int J Biol Macromol. 2023; 230: 123189. doi:10.1016/j.ijbiomac.2023.123189

[20] Alipoor B, Ghaedi H, Meshkani R, et al. Association of MiR-146a expression and type 2 diabetes mellitus: A meta-analysis. Int J Mol Cell Med. 2017; 6(3): 156-163. doi:10.22088/acadpub.BUMS.6.3.156

[21] Taganov, K.D., Boldin, M.P., Chang, J., & Baltimore, D. (2006). NF-kappaB-dependent induction of microRNA miR-146a, an inhibitor targeted to the IRAK1/TRAF6 signaling complex. Genes & Development, 20(18), 2233–2243. This study identifies the role of miRNA-146a in regulating inflammation and immune responses, particularly through its modulation of the IRAK1/TRAF6 signaling complex, which is integral to NF-kB activation and subsequent inflammatory cytokine production

[22] Sharma, B., Kumar, A., & Sarin, J. (2016). Academic stress, anxiety, remedial measures adopted and its satisfaction among medical students: A systematic review. International Journal of Health Sciences and Research, 6, 368-376. This reference focuses on the systematic review regarding stress and its effects on medical students, which might be unrelated to your study's focus on miRNAs and cortisol but provides useful context in educational stress

[23] Maratni NPT, Saraswati MR, Ayu Dewi NN, Suastika K. MIRNA146a And Diabetes-Related Complications: A Review. Curr Diabetes Rev. 2023; 19(9): e141022209958. doi: 10.2174/1573399819666221014095715

[24] Caggiu E, Paulus K, Mameli G, Arru G, Sechi GP, Sechi LA. Differential expression of miRNA 155 and miRNA 146a in Parkinson's disease patients. eNeurologicalSci. 2018; 13:1-4. doi: 10.1016/j.ensci.2018.09.002

[25] Fan W, Liang C, Ou M, Zou T, Sun F, Zhou H, Cui L. MicroRNA-146a Is a Wide-Reaching Neuroinflammatory Regulator and Potential Treatment Target in Neurological Diseases. Front Mol Neurosci. 2020; 13:90. doi: 10.3389/fnmol.2020.00090

[26] Zhang X, Deng X, Zhou J, et al. The association of serum cortisol level with microalbuminuria in patients with type 2 diabetes and prediabetes. Int J Med Sci. 2020; 17(18): 2998-3004. doi:10.7150/ijms.48742

[27] Balasubramanyam M, Aravind S, Gokulakrishnan K, Prabu P, Sathishkumar C, Ranjani H, Mohan V. Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes. Mol Cell Biochem. 2011 May;351(1-2):197-205. doi: 10.1007/s11010-011-0727-3. Epub 2011 Jan 20. PMID: 21249428.

[28] Janssen JAMJL. New Insights into the Role of Insulin and Hypothalamic-Pituitary-Adrenal (HPA) Axis in the Metabolic Syndrome. Int J Mol Sci. 2022; 23(15): 8178. doi: 10.3390/ijms23158178

[29] Steffensen C, Dekkers OM, Lyhne J, Pedersen BG, Rasmussen F, Rungby J, Poulsen PL, Jørgensen JOL. Hypercortisolism in Newly Diagnosed Type 2 Diabetes: A Prospective Study of 384 Newly Diagnosed Patients. Horm Metab Res. 2019; 51(1): 62-68. doi: 10.1055/a-0809-3647

[30] Mohan A, Saxena S, Kaur A, Ali W, Akduman L. Serum cortisol is a biomolecular biomarker for severity of diabetic retinopathy. Mol Vis. 2021; 27:429-437

[31] Esguerra JLS, Ofori JK, Nagao M, Shuto Y, Karagiannopoulos A, Fadista J, Sugihara H, Groop L, Eliasson L. Glucocorticoid induces human beta cell dysfunction by involving riborepressor GAS5 LincRNA. Mol Metab. 2020; 32: 160-167. doi: 10.1016/j.molmet.2019.12.012

[32] Sun B, Chen H, Xue J, Li P, Fu X. The role of GLUT2 in glucose metabolism in multiple organs and tissues. Mol Biol Rep. 2023; 50(8): 6963-6974. doi: 10.1007/s11033-023-08535-w

[33] Zhang X, Deng X, Zhou J, Qiu K, Deng M, Lin Z, Mosha SS, Li W. The Association Of Serum Cortisol Level With Microalbuminuria In Patients With Type 2 Diabetes And Prediabetes. Int J Med Sci. 2020; 17(18): 2998-3004. doi: 10.7150/ijms.48742

[34]- Sun S, Wang Y. Relationship between cortisol and diabetic microvascular complications: a retrospective study. Eur J Med Res. 2023 Sep 29;28(1):391. doi: 10.1186/s40001-023-0132

[35]- Joseph JJ, Golden SH. Cortisol dysregulation: the bidirectional link between stress, depression, and type 2 diabetes mellitus. Ann N Y Acad Sci. 2017 Mar;1391(1):20-34. doi: 10.1111/nyas.13217. Epub 2016 Oct 17. PMID: 27750377; PMCID: PMC5334212.

[36] Reynolds, R. M., Labad, J., Strachan, M. W., Braun, A., Fowkes, F. G., Lee, A. J., Frier, B. M., Seckl, J. R., Walker, B. R., & Price, J. F. (2010). Elevated Fasting Plasma Cortisol Is Associated with Ischemic Heart Disease and Its Risk Factors in People with Type 2 Diabetes: The Edinburgh Type 2 Diabetes Study. The Journal of Clinical Endocrinology & Metabolism, 95(4), 1602-1608. https://doi.org/10.1210/jc.2009-2112

[37] Diabetes UK. Stress and diabetes: How stress affects your emotional and physical wellbeing [Internet]. Available from: https://www.diabetes.org.uk/living-with-diabetes/emotional-wellbeing/stress?utm_source=chatgpt.com

[38] Pittas, A. G., Lau, J., Hu, F. B., & Dawson-Hughes, B. (2007). The Role of Vitamin D and Calcium in type 2 diabetes. A systematic Review and Meta-Analysis. The Journal of Clinical Endocrinology and Metabolism, 92(6), 2017. https://doi.org/10.1210/jc.2007-0298

[39] Palomer X, González-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab. 2008 Mar;10(3):185-97. doi: 10.1111/j.1463-1326.2007.00710.x. PMID: 18269634.

[40] Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. Nutrients. 2013 Sep 13;5(9):3605-16. doi: 10.3390/nu5093605. PMID: 24067388; PMCID: PMC3798924.
[41] Luo Y, Cui C, Han X, Wang Q, Zhang C. The role of miRNAs in polycystic ovary syndrome with insulin resistance. J Assist Reprod Genet. 2021 Feb;38(2):289-304. doi: 10.1007/s10815-020-02019-7. Epub 2021 Jan 6. PMID: 33405004; PMCID: PMC7884539.
[42] Li, M., Chi, X., Wang, Y. et al. Trends in insulin resistance: insights into mechanisms and therapeutic strategy. Sig Transduct Target Ther 7, 216 (2022). https://doi.org/10.1038/s41392-022-01073-0
[43] Zhao X, An X, Yang C, Sun W, Ji H, Lian F. The crucial role and mechanism of insulin resistance in metabolic disease. Front Endocrinol (Lausanne). 2023 Mar 28;14:1149239. doi: 10.3389/fendo.2023.1149239. PMID: 37056675; PMCID: PMC10086443.
[44] Rasoulinejad SA, Akbari A, Nasiri K. Interaction of miR-146a-5p with oxidative stress and inflammation in complications of type 2 diabetes mellitus in male rats: Anti-oxidant and anti-inflammatory protection strategies in type 2 diabetic retinopathy. Iran J Basic Med Sci. 2021 Aug;24(8):1078-1086. doi: 10.22038/IJBMS.2021.56958.12706. PMID: 34804425; PMCID: PMC8591764.
[45] Clayton SA, Jones SW, Kurowska-Stolarska M, Clark AR. The role of microRNAs in glucocorticoid action. J Biol Chem. 2018; 293(6): 1865-1874. doi: 10.1074/jbc.R117.000366
[46] Ghaffari M, Razi S, Zalpoor H, Nabi-Afjadi M, Mohebichamkhorami F, Zali H. Association of MicroRNA-146a with Type 1 and 2 Diabetes and their Related Complications. J Diabetes Res. 2023; 2023:2587104. doi: 10.1155/2023/2587104
[47] Zgheib C, Hilton SA, Dewberry LC, Hodges MM, Ghatak S, Xu J, Singh S, Roy S, Sen CK, Seal S, Liechty KW. Use of Cerium Oxide Nanoparticles Conjugated with MicroRNA-146a to Correct the Diabetic Wound Healing Impairment. J Am Coll Surg. 2019; 228(1): 107-115. doi: 10.1016/j.jamcollsurg.2018.09.017
[48] Chen H, Lan HY, Roukos DH, Cho WC. Application of microRNAs in diabetes mellitus. J Endocrinol. 2014; 222(1): R1-R10. doi: 10.1530/JOE-13-0544
[49] Council for International Organizations of Medical Sciences and WHO. International Ethical Guidelines for Health-related Research Involving Humans. Geneva; 2016.
[50] Metwally AM, Amer HA, Salama HI, Abd El Hady SI, Alam RR, Aboulghate A, Mohamed HA, Badran HM, Saadallah AA, El-Sonbaty MM, Eltahlawy E. Egyptian patients'/guardians' experiences and perception about clinical informed consent and its purpose: Cross sectional study. PloS one. 2021 Jun 14;16(6):e0252996