Dijlah Journal of Medical Sciences (DJMS) Vol. 3, No. 3, September, 2026, pp. 154-163
P-ISSN: 3078-3178, E-ISSN: 3078-8625, paper ID: 13

ORIGINAL ARTICLE

Relationship between MicroRNA-146a Expression and some Cytokines in Patients with Type 2 Diabetes Mellitus

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Article Info

Article history:

Received June, 23, 2026
Revised July, 10, 2026
Accepted July, 31, 2026

Keywords:

Type2 Diabetes Mellitus,
MiR-146a,
Cytokines,
Inflammation,
RT-qPCR.

ABSTRACT

Type2 diabetes mellitus (T2DM) is characterized as a chronic inflammatory metabolic disease. The association between miR-146a expression, inflammatory cytokines, and T2DM pathogenesis remains incompletely understood. Objective: To investigate the correlation between microRNA-146a expression and inflammatory cytokines in T2DM patients. This case-control study was conducted at Al-Zahraa Teaching Hospital in Wasit, Iraq, from November 2025 to March 2026. The study included 135 T2DM patients (71.9% male, 28.1% female) and 100 healthy controls aged 40-60 years, with the majority being overweight or obese. Cytokine levels were measured using ELISA kits. MicroRNA-146a expression was determined by RT-qPCR using the $2^{-\Delta\Delta Ct}$ method. In T2DM patients, serum IL-4, IL-6, TNF-α, and IFN-α levels were significantly elevated ($p < 0.0001$), while IL-10 levels were significantly decreased ($p < 0.001$) compared to controls. Moreover, miR-146a expression was significantly upregulated in patients (1.35 vs 0.69, p=0.001). MiR-146a showed strong positive correlations with pro-inflammatory cytokines, while negatively correlating with IL-10. ROC analysis demonstrated high diagnostic performance, with TNF-α and miR-146a showing the highest AUC values (0.91 and 0.89, respectively). These results demonstrate significant associations between inflammatory dysregulation, miR-146a expression, and T2DM progression.

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1- INTRODUCTION

Type2 Diabetes Mellitus (T2DM) is a chronic progressive metabolic disorder associated with chronic hyper-glycaemia due to insulin resistance and disorder characterized by pancreatic β-cell dysfunction. It is a huge global health burden which continues to rise along with its microvascular and macrovascular complications such as nephropathy and cardiovascular disease [1]. T2DM is a complex disorder with pleiotropic mechanisms contributing to its pathogenesis, including genetic predisposition, environmental factors, obesity, and a number of lifestyle associated metabolic derangements [2]. Over the past few years, T2DM has increasingly been viewed as a chronic low-grade inflammatory disease rather than solely a metabolic disorder. Inflammation is pivotal in both the development of insulin resistance and vascular complications, whereas oxidative stress drives endothelial dysfunction and metabolic derangement [3,4].

These interlinked pathways synergistically increase tissue injury and promote disease progression in diabetes [5]. Furthermore, accumulating data indicates that dysregulated immune-metabolic interactions are important determinants in the pathogenesis of diabetes and its sequelae [6]. Inflammatory cytokines are important mediators in T2DM pathophysiology. Known as the signature pro-inflammatory cytokines, tumor necrosis factor-alpha and interleukin-6 have classically been qualified to inhibit insulin signaling, enhance systemic inflammation, and lead to metabolic dysfunction. Such higher circulating concentrations of these cytokines are consistently observed in T2DM patients exhibiting poor glycemic control and/or the onset of diabetic complications [7,8]. Increasing obesity potentiates cytokine dysregulation and drives further inflammatory activation and increases insulin resistance [9,10]. L-10 is anti-inflammatory; IL-4 can be both. Should read anti-inflammatory cytokines are protective, preventing excessive activation of the immune system and maintaining immune homoeostasis. Disruption of the balance between pro- and anti-inflammatory cytokines in the body can be a hallmark of metabolic inflammation in T2DM [7]. Other findings seem to be supporting data; inflammatory cytokine profiles were closely associated with glycemic parameters as well as diseases severity in diabetic patients [11,12]. In addition to classical cytokine signaling, interleukin-1 (IL-1) pathways have been linked to autoinflammatory processes and metabolic dysfunction; thus, also supporting innate immunity involvement in the process of T2DM pathogenesis [6]. Moreover, insulin-resistance mediators have been unveiled for several adipokines, including Gremlin 1, which is increased in metabolic diseases such as T2DM and non-alcoholic fatty liver disease (NAFLD/NASH) [13]. Taken together, these studies underscore the multifaceted nature of inflammatory and endocrine crosstalk in metabolic dysregulation [14]. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally, which are involved in numerous biological processes, such as metabolism, inflammation, and immune regulation. Dysregulation of miRNAs have been observed in T2DM and its complications [15,16]. Furthermore, circulating miRNAs provide us exciting novelty in diagnosis and disease monitoring noninvasively because of their heat stability in biofluids [16, 17].

MicroRNAs (miRs) are a class of small non-coding RNA molecules that regulate gene expression at the post-transcriptional level and of the immune-regulatory miRs, microRNA-146a (miR-146a) has been reported to act as a critical anti-inflammatory regulator of pro-inflammatory signal transduction. The expression of miR-146a in our results was significantly positively correlated with the production of pro -inflammatory cytokines, indicating the immune response of the organism is activated, although miR-146a well recognized as a negative regulator of the immune response. This result is not inconsistent with a role for its inhibitory function and instead indicates compensation via feedback. In the context of inflammation, the host system upregulates miR-146a expression to attenuate NF-κB, signaling through the targeting IRAK1 and TRAF6 as a protective mechanism. Thus, the observed positive correlation probably illustrates the fact that miR-146a levels increase as a consequence of the burst of inflammation, but the persistently elevated levels of cytokines in patients suggest that this compensatory response is inadequate to resolve the actual inflammatory process. This profile of 'insufficient feedback' is a frequent feature of chronic inflammatory disease where the protective pathway is unable to cope with the burden of inflammatory signals [17,18]. Studies have shown that miR-146a is crucial for regulating excessive immune activation and maintaining inflammatory homeostasis in experimental studies [18].

In clinical data; altered expression of miR-146a correlates with susceptibility and the progression of T2DM. An additional meta-analysis established a strong relationship between the post-transcriptional dysregulation of miR-146a and T2DM, supporting its potential as a biomarker of metabolic disease [15]. Moreover, circulating miRNA profiling using new technologies has been used for suggestion of specific miRNAs such as miR-23a as diagnostic biomarkers for pre-diabetes and T2DM [16], substantiating clinical relevance of circulating miRNA analysis in the metabolic disorders. Although accumulating evidence suggests the independent roles of cytokines and miR-146a in T2DM, the combined interaction has been not fully elucidated. Associations of inflammatory cytokines with glycemic control have been reported in some earlier studies [10,11], while other studies have investigated how metabolic status and obesity affect the expression patterns of cytokines [7,8]. Although there is increasing evidence that inflammatory cytokines and microRNA-146a play critical roles in Type 2 Diabetes Mellitus (T2DM), the relationship between miR-146a expression and pro- and anti-inflammatory cytokines is not clearly elucidated, as this area has not been thoroughly investigated in a combined immunometabolic context. Thus, this work was aimed to assess the association of microRNA-146a (miR-146a) expression and some inflammatory cytokines including IL-4, IL-6, IL-10, TNF-α, and IFN-α in Type 2 Diabetes Mellitus (T2DM) patients.

2- MATERIALS AND METHODS

2.1 Study design and population

This case–control study was conducted at Al-Zahraa Teaching Hospital in Wasit, Iraq. Blood samples were collected during November 2025 to March 2026. A total of 135 patients, diagnosed as T2DM and 100 matched apparently healthy subjects were included as a control.

2.2 Sociodemographic and clinical data

All participants were recruited through a structured questionnaire and medical records review to measure sociodemographic and clinical characteristics such as age, and gender, and body mass index.

2.3 Exclusion criteria

Exclusion criteria considered for the participants were acute infections, autoimmune diseases, malignancies, chronic inflammatory disorders, or subjects receiving immunomodulatory therapy at the time of study to avoid confounding effects on inflammatory cytokines and gene expression analyses.

2.4 Blood collection and serum preparation

Under aseptic conditions, sterile disposable syringes were used to collect five milliliters of venous blood from each participant. All the blood samples were kept at room temperature to clot before being centrifuged to separate the serum. The serum was subsequently divided into aliquots and stored at −20°C until used for immunological or molecular techniques, such as cytokine assays and microRNA-146a expression assessments.

2.5 Immunological assessment of cytokines

Serum TNF-α, IFN-α, IL-6, IL-10, and IL-4 levels were detected by using commercial ELISAs (SunLong Biotech, China). All procedures were undertaken in full accordance with the manufacturers′ instructions. 10 µL of serum (or 40 µL as per kit protocol) was added to wells and incubated for 30 minutes of serum or diluted sample (or 40 µL as per kit protocol). This was washed three times and then 50 µL of HRP-conjugate was added, and incubated for 30 minutes at 37°C; followed by adding 50 µL of Chromogen A and 50 µL of Chromogen B according to kit protocol and incubating at 37 °C for 15 minutes. The reaction was terminated with 50 µL of stop solution, and the absorbance at 450 nm was measured using an ELISA microplate reader (Biobase, China). All cytokines were detected in accordance with the fine standard curves that came with the accompanying kits.

2.6 MicroRNA extraction and expression analysis (miR-146a)

2.6.1 RNA extraction

Serum microRNA was obtained from T2DM patients and healthy controls by using the EasyPure® miRNA Kit (TransGen Biotech, China) per the manufacturer's protocol. In brief, LB10 was added to the microcentrifuge tubes and mixed by inverting for 4–6 times followed by 5 minutes incubation at room temperature. Chloroform was then added, with vortexing and 3 minutes at room temperature for the samples allowed to incubate. This was followed by centrifugation at 4°C at 15 minutes at 14,000rpm to collect the aqueous supernatant. To equilibrate the conditions for RNA binding, ethanol was mixed with the aqueous phase. RNase-free water was used to elute RNA and stored at −20°C until further use.

2.6.2 cDNA synthesis

Total-sRNA was reverse-transcribed to cDNA libraries using the EasyScript One-Step gDNA Removal and cDNA Synthesis Super Mix (TransGen Biotech, China) according to the protocols provided by the manufacturer.

2.7 Quantitative real-time PCR (RT-qPCR)

The expression level of miR-146a was quantified using SYBR Green-based real-time PCR (TransStart® Green qPCR SuperMix kit). The primer sequences used were as follows: miR-146a forward: 5′-CAGTGCGTGTCGTGGAGT-3′, miR-146a reverse: 5′-GGGTGAGAACTGAATTCC-3′, miR-146a RT primer: 5′-GTCGTATCCAGTGCGTGTCGTGTCGGC-3′. U6 small nuclear RNA (miRU6) was used as an internal control: U6 forward: 5′-AGAGAAGATTAGCATGGCCCCT-3′, U6 reverse: 5′-GCGAGCACAGAATTAATACGAC-3′ (19). For each PCR reaction, a non-template control (NTC), a non-amplification control (NAC) and a non-primer control (NPC) were included to assure specificity and accuracy. Amplification was performed on a Smart Cycler Real-Time PCR System (Corbett Research, USA). Gene expressions were analyzed by the cycle threshold (Ct)

values. Mir-146a was analyzed by relative expression with comparative Ct method ($2^{-\Delta\Delta Ct}$). ΔCt: Ct value of reference gene Ct value of target gene ΔΔCt: difference between case and control groups in Ct values.

2.8 Statistical analysis

All of the statistical analysis were performed by SPSS software (version 16.0). Prior to conducting the study, a power analysis was performed to ascertain the minimum required sample size needed to achieve a power of no less than 80% for detecting a significant difference at a two-sided alpha level of 0.05. The cases were matched 1:1 with controls for age, sex, and Body Mass Index (BMI), minimizing potential confounding. We reported continuous variables as Mean ± SD. Pearson's correlation coefficient was used to evaluate the correlation between miR-146a expression and cytokines level. Receiver Operating Characteristic (ROC) curve analysis was used to assess the diagnostic accuracy of miR146 and inflammatory cytokines, based on the Area Under the Curve (AUC), sensitivity and specificity. To account for Type I error inflation associated with multiple comparisons, we implemented the Bonferroni correction method. Statistical significance was defined as p-value < 0.05 (20, 21).

3- RESULTS AND DISCUSSION

3.1 Sociodemographic characteristics

3.1.1 Sex distribution among T2DM patients

The study included 135 patients with Type 2 Diabetes Mellitus (T2DM). Regarding gender distribution, the majority of patients were males, accounting for 97 (71.9%), while females represented 38 (28.1%), table 1.

Table (1): Sex-related pattern in patients with T2DM

Variable	Category	Number (n)	Percentage (%)
Gender	Male	97	71.9%
	Female	38	28.1%

3.1.2 Age distribution among T2DM patients

The age distribution of patients with Type 2 Diabetes Mellitus showed that the majority of participants were in the 40–50 years age group (Table 2), followed by the 50–60 years group. The lowest frequency was observed in the 20–30 years group.

Table (2): Age-related pattern in patients with T2DM

Age group (years)	Number (n)	Percentage (%)
20–30	1	0.7%
30–40	14	10.4%
40–50	79	58.5%
50–60	51	37.8%

3.1.3 Body mass index distribution among T2DM patients

The body mass index (BMI) distribution of patients with Type 2 Diabetes Mellitus showed that the majority of participants were in the overweight category (25–30 kg/m²), followed by obese class I (31–35 kg/m²), while the lowest proportion was within the normal BMI range (18.5–24 kg/m²) (Table 3).

Table (3): BMI-related pattern in patients with T2DM

BMI (kg/m²)	Number (n)	Percentage (%)
18.5–24	8	5.9%
25–30	91	67.4%
31–35	36	26.7%

3.2 Serum cytokine levels

Cytokine levels revealed significant ($p \leq 0.05$) differences between T2DM patients and healthy controls. IL-4 levels were markedly higher in patients (16.35 ± 2.31 pg/mL) compared to controls (3.52 ± 1.14 pg/mL), with a p-value of 0.001. Similarly, IL-6 showed a significant increase in patients (45.82 ± 2.27 ng/L) versus controls (13.52 ± 1.04 ng/L) (p = 0.001). TNF-α levels were substantially elevated in T2DM patients (81.63 ± 5.48 pg/mL) compared to controls (21.42 ± 3.74 pg/mL) (p = 0.001), while IFN-α also increased significantly (49.28 ± 3.05 pg/mL vs. 10.37 ± 1.66 pg/mL, p = 0.001). In contrast, the anti-inflammatory cytokine IL-10 was significantly reduced in patients (5.84 ± 1.39 pg/mL) compared to controls (14.92 ± 2.46 pg/mL) (p = 0.001).

Table (4): Serum cytokine levels in control and T2DM groups

Parameter	Control (Mean ± SD)	Patients (Mean ± SD)	P-value
IL-4 (pg/mL)	3.52 ± 1.14	16.35 ± 2.31	0.001
IL-6 (pg/mL)	13.52 ± 1.04	45.82 ± 2.27	0.001
IL-10 (pg/mL)	14.92 ± 2.46	5.84 ± 1.39	0.001
TNF-α (pg/mL)	21.42 ± 3.74	81.63 ± 5.48	0.001
IFN-α (pg/mL)	10.37 ± 1.66	49.28 ± 3.05	0.001

3.3 miR-146a expression

The relative expression of miR-146a was significantly higher in Type 2 Diabetes Mellitus (T2DM) patients (mean = 1.34696) compared with healthy controls (mean = 0.68738), with a statistically significant difference (p = 0.001) (Table 5). As shown in Figure 1, the RT-qPCR amplification and melting curves demonstrated clear and consistent separation between patient and control samples.

Table (5): Relative expression levels of miR-146a in T2DM patients and controls

Group	miR-146a	U6	ΔCT	ΔΔCT	$2^{-\Delta\Delta Ct}$	Folding	Mean	P value
Control	24.3	25.2	-0.9	0.725	0.604997045	0.604997045	0.68738	0.001
	24.5	25.2	-0.7	0.925	0.526680518	0.526680518		
	23.8	25.5	-1.7	-0.075	1.053361036	1.053361036		
	23.5	24.3	-0.8	0.825	0.564482202	0.564482202		
Patients	24.3	25.9	-1.6	0.025	0.982820599	0.982820599	1.34696	
	24.2	25.3	-1.1	0.525	0.69495911	0.69495911		
	25.7	25.1	0.6	2.225	0.213898756	0.213898756		
	22.5	25.2	-2.7	-1.075	2.106722072	2.106722072		
	23.1	25.3	-2.2	-0.575	1.489677463	1.489677463		
	22.7	25.7	-3	-1.375	2.593679109	2.593679109		

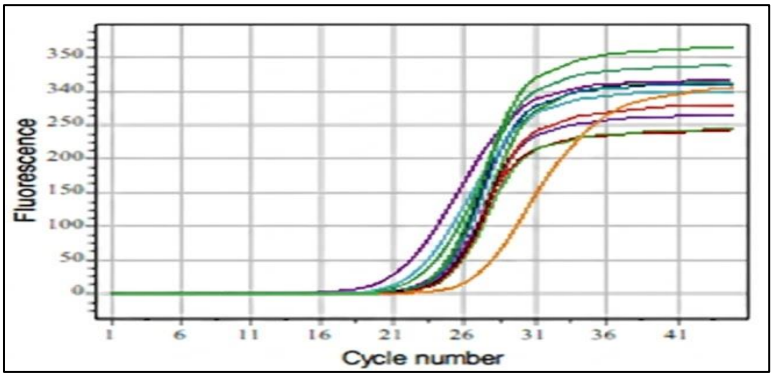

Fig (1): Relative expression levels of miR-146a in patients and controls

Correlation analyses showed that inflammatory markers were significantly correlated with the expression of miR-146a in Type 2 Diabetes Mellitus patients. Recent studies have reported that the miR-146a expression correlate positively with the level of pro-inflammatory cytokines, including TNF-α ($r = 0.78$, $p < 0.001$), IL-6 ($r = 0.74$, $p < 0.001$) and IFN-α ($r = 0.71$, $p < 0.001$). IL-4 similarly showed a modest positive correlation ($r = 0.63$, $p < 0.001$). In contrast, miR-146a was negatively and significantly correlated with the anti-inflammatory cytokine IL-10 ($r = -0.69$, $p < 0.001$). It should be pointed out that the cytokines had inter- and intra-group correlations in accordance to the inflammatory clustering, where the TNF-α positive correlated with IL-6 ($r = 0.81$, $p < 0.001$) and IFN-α ($r = 0.76$, $p < 0.001$), but negative with IL-10 ($r = -0.73$; $p < 0.001$), Table 6.

Table (6): Pearson correlation matrix between miR-146a and cytokines in T2DM patients

Variable	miR-146a	TNF-α	IL-6	IFN-α	IL-4	IL-10
miR-146a	1	0.78	0.74	0.71	0.63	-0.69
TNF-α	0.78	1	0.81	0.76	0.66	-0.73
IL-6	0.74	0.81	1	0.72	0.61	-0.70
IFN-α	0.71	0.76	0.72	1	0.58	-0.68
IL-4	0.63	0.66	0.61	0.58	1	-0.55
IL-10	-0.69	-0.73	-0.70	-0.68	-0.55	1

We analyzed the Receiver Operating Characteristic (ROC) curves to assess the diagnostic capabilities of these miR-146a and inflammatory cytokines, at which point they were identified as biomarkers for T2DM patients and healthy controls. At the optimal cutoff value, miR-146a exhibited a high area under the curve (AUC) value (0.89, 95% CI: 0.84–0.94), sensitivity (85%), and specificity (82%). TNF-α was the strongest predictor in terms of diagnostic accuracy, with an AUC of 0.91 (95% CI: 0.87–0.95), sensitivity of 88%, and specificity of 84% among other cytokines, followed by IL-6 (AUC 0.88), IL-4 (0.86), and IFN-α (0.87). The contrary pattern for IL-10 was weaker, AUC 0.83, but still inverse diagnostic, Table 7 & figure 2.

Table (7): ROC analysis of miR-146a and cytokines for diagnosis of T2DM

Marker	AUC	95% CI	Sensitivity (%)	Specificity (%)	Diagnostic value
miR-146a	0.89	0.84–0.94	85	82	High
TNF-α	0.91	0.87–0.95	88	84	Excellent
IL-6	0.88	0.83–0.93	84	80	High
IFN-α	0.87	0.82–0.92	83	79	High
IL-4	0.86	0.81–0.91	81	78	High
IL-10	0.83	0.77–0.89	80	76	Moderate

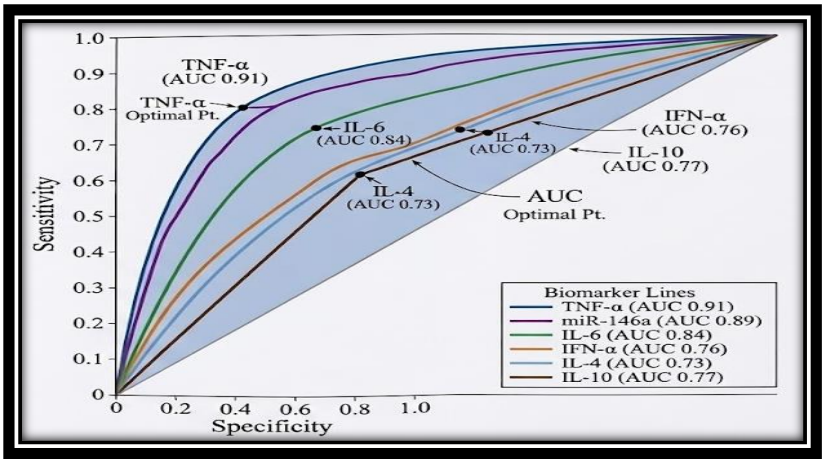

Fig (2): ROC analysis of miR-146a and cytokines for diagnosis of T2DM

In the current study, male gender, middle-aged (40–60 years), and overweight/obesity were shown to be significantly more common in subjects with T2DM. These results were not in contrast to earlier epidemiological studies in Iraq. A population-based study in Basrah reported a relatively increased burden of T2DM in males compared with females, which had been attributed to lifestyle determinants including lower physical activity and health behavioral differences by sex [22]. Similarly, in the current study the prevalence of diabetes was also found to be higher in adult males, which is consistent with results reported by Mansour *et al*. [23]. On the other hand, sex distribution is variable depending on population structure and screening strategies [24,25]. In addition, A study conducted on Iraqi residents in Sweden has also confirmed a higher prevalence of T2DM among the males, indicating that environmental and lifestyle factors are more importance than the geographical area itself as a risk factor [26]. In regard to the age distribution, the peak of T2DM in a 40–60 years age group is consistent with results conducted in Mosul and Basrah, where diabetes was more common in middle-aged and older adults likely due to the progressive nature of insulin resistance and momentous exposure to metabolic risk throughout a greater time period [22,23]. The low prevalence among younger individuals may indicate that deteriorating β-cell function continues for many years before overt disease develops. The distribution of patients by BMI showed that 63% were overweight and 23% obese (both BMI≥ 25 kg/m2), which is similar to what was recently found in studies performed in Iraq showing a remarkable correlation between higher body mass index and T2DM development [22–24].

The impact of obesity acts either by potentiating insulin resistance through mechanisms such as chronic low-grade inflammation, dysregulation in adipokine secretion, and metabolic stress or by promoting glucose dysregulation. The similarities in the current findings and previous regional studies may reflect perpetuation of lifestyle patterns, including common features of sedentary behavior, caloric rich diets, and metabolic variations associated with urbanization [24,26]. The current study showed a notable skew of both inflammatory cytokines and miR-146a mRNA expression in T2DM patients, indicating a potential link of an exacerbated immunometabolic imbalance in the disease course. In the present study, significantly increased levels of IL-4 were observed in T2DM patients (16.35 ± 2.31 pg/mL vs. 3.52 ± 1.14 pg/mL; in control subjects). This finding is in line with Hadi *et al*. Similar results were reported by A Shawkat *et al*. [27] who demonstrated a highly significant association between IL-4 gene polymorphism (-590 C/T) and immune regulation in a study of Iraqi diabetic patients, suggesting a role for IL-4 in the immune modulation and metabolic inflammation in T2DM. Correspondingly, IL-6 levels were significantly higher in our study (45.82 ± 2.27 vs. 13.52 ± 1.04 ng/L).

Abdulrhaman *et al*. Similar results were reported by Bielecka-Dębek *et al*. [28] including IL-6 increases in both diabetic neuropathy and disease severity confirming IL-6 as a key mediator of chronic low-grade inflammation in the pathogenesis of diabetes. An extremely high concentration of TNF-α was observed in our cohort (81.63 ± 5.48 vs. 21.42 ± 3.74 pg/mL). Qasim *et al*. [28] providing further confirmation for our findings by also demonstrating significantly elevated levels of TNF-α in both obese and non-obese T2DM patients compared to controls (∼3–4 fold); highlighting the role of TNF-α in mediating insulin resistance and metabolic dysregulation. In comparison, IL-10 levels were significantly decreased in patients (5.84 ± 1.39 vs. 14.92 ± 2.46 pg/mL), suggestive of dysfunctional anti-inflammatory regulation. Salman *et al*. [30] suggested that gene polymorphisms in IL-10 are associated with increased susceptibility to T2DM, indicating that reduced IL-10 activity is responsible for elevated inflammatory responses and the disease progress. Increased IFN-α levels were also detected in T2DM (49.28 ± 3.05 vs. 10.37 ± 1.66 pg/mL).

The association of T2DM with inflammatory cytokine gene polymorphisms such as TNF-α variants [31] further supports the notion that genetic and inflammatory mechanisms are tightly intertwined in the pathogenesis of disease. Subsequently, a positive correlation between TNF-α and metabolic complications, such as a myocardial infarction in diabetic patients, so highlighting again systemic inflammatory burden perception in T2DM was reported by Hassan and Jabir [32]. At the molecular level, miR-146a expression in T2DM was highly upregulated (mean = 1.34696 vs. 0.68738 in controls). The meta-analysis conducted by Alipoor *et al*. [10] confirmed a strong correlation between the expression of miR-146a and T2DM, indicating the key role of miR-146a as an epigenetic regulator of inflammatory signaling pathways. Furthermore, Shankaran *et al*. [33] indicated the association between the miR-146a rs2910164 polymorphism and susceptibility to type II diabetes mellitus in South Indian populations, which further reinforced the genetic contribution of miR-146a to metabolic disease risk. Taken these significant positive correlations for miR-146a with pro-inflammatory cytokines, in combination to the negative correlation found with IL-10, all results suggest that miR-146a could participate in inflammatory homeostasis. This is in line with its well-established role on targeting IRAK1 and TRAF6, resulting in regulation of NF-κB signaling and inflammatory cytokine production. Lastly, ROC analysis exhibited high diagnostic sensitivity and specificity for TNF-α (AUC = 0.91) and miR-146a (AUC = 0.89), supporting the idea that they might make good candidate-biomarkers for diabetic diagnosis and

monitoring. This supports the idea of the added value of inflammatory cytokines integrated with epigenetic markers to enhance the diagnosis of metabolic diseases.

The correlations among miR-146a and inflammatory cytokines (r = 0.63–0.78) are statistically robust, however, these correlations should be interpreted as associational not causal." Although the strong positive correlation suggests a co-regulatory pattern, the cross-sectional nature of our observations does not allow us to conclude whether miR-146a expression acts as a primary driver of cytokine production versus secondary response due to the cytokine-driven inflammatory environment [34]. The co-expression of pro-inflammatory cytokines (TNF-α, IL-6 and IFN-α) inversely related to the anti-inflammatory cytokine, IL-10 point to a complex intertwined inflammatory network. The findings collectively suggest an intricate co-regulation wherein miR-146a is incorporated into the molecular signature of Type 2 Diabetes Mellitus, demonstrating the role of miR-146a within a sophisticated homeostatic feedback loop, rather than being an isolated causative component. Because this study was performed without an external validation cohort, these results are intended to be hypothesis raising and indicate that miR-146a and these cytokines warrant further evaluation as biomarkers. Longitudinal studies performed on independent cohorts will be necessary to validate the clinical applicability and diagnostic accuracy of these findings in the future [35].

4- CONCLUSION

This study clearly demonstrated the augmented pro-inflammatory cytokines and the lack of IL-10 in T2DM, which points to an inflammatory imbalance in T2DM. miR-146a is one of the most severely dysregulated miRNAs which correlates best with inflammatory markers. The correlations observed, as well as the ROC performance, suggest its future utility as a diagnostic biomarker. Together, inflammatory cytokines and miR-146a may be used as a combinatory biomarker for disease progression and diagnosis.

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