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Association of mirna-29a expression and HSV-1 infection in type 2 diabetes mellitus

Nael shakir Mohamed[1*], Dunya Fareed Salloom[2]

[1,2]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*

Corresponding author: Nael shakir Mohamed (*Email: naael.shaker2402m@sc.uobaghdad.edu.iq*)

Abstract

To investigate the association between miR-29a expression, HSV-1, TNF-α infection, and type 2 diabetes mellitus (T2DM). A case-control study was conducted on 89 T2DM patients and 88 healthy individuals, analyzing miR-29a gene expression using RT-qPCR and assessing anti-HSV-1 IgG antibody and TNFα serum level via ELISA technique. They revealed a significant upregulation of miR-29a in T2DM patients ($P < 0.0001$), indicating a link between inflammation and diabetes progression, and significant higher levels of TNF-α in T2DM patients ($P < 0.0001$), indicated the pathologic role in metabolic dysfunction and a positive correlation with insulin resistance. However, HSV-1 IgG seropositivity showed no significant association with T2DM ($P > 0.05$), suggesting that prior HSV-1 exposure may not be a reliable biomarker for diabetes risk. ROC analysis demonstrated high sensitivity (1.0) but low specificity (0.023), highlighting the need for improve diagnosing models. These findings underscore the considerably of miR-29a as a potential biomarker for T2DM and emphasize the role of inflammatory pathways in illness progression.

Keywords: Anti-HSV-1 IgG antibody, HbA1c, RT-qPCR, miR-29a, T2DM, fasting blood glucose, TNF-α, Type 2 diabetes mellitus.

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1. Introduction

Type 2 diabetes mellitus (T2DM) represents a complex metabolic disorder categorized by insulin resistance and pancreatic β-cell dysfunction. While genetic predisposition and lifestyle factors persist determinants of T2DM pathogenesis, emerging research highlights the potential involvement of viral infections particularly Herpes Simplex Virus type 1 (HSV-1) infection as a contributing factor [1]. This association may arise through the viral-induced alterations in metabolic and inflammatory pathways.

Current studies have identified microRNAs (miRNAs) like critical regulators in metabolic diseases. These small non-coding RNA molecules, typically 18 – 22 nucleotides in length, exert post-transcriptional control over gene expression networks. The miRNA29 family (comprising miRNA-29a, -29b, and -29c isoforms) has drawn particular research interest due to its dual role in modulating both glucose homeostasis and antiviral immune responses. Scientist's established that miRNA-29 directly influences insulin sensitivity through targeted regulation of key insulin signaling pathway components [2]. Particularly, these miRNA molecules appear to function at the intersection of viral pathogenesis and metabolic dysregulation [3].

The biological consequence of miRNA broadens beyond metabolic regulation. These molecules are involved in different physiological processes, including cellular differentiation, programmed cell death, and host defense mechanisms. Emerging evidence positions miRNA-29 as a molecular bridge connecting HSV-1 [4]. Moreover, dysregulated miRNA expression patterns have been implicated across multiple disease states, present Alzheimer's, Parkinson's, and Huntington's diseases, autoimmune disorders, and cerebrovascular accidents [5, 6]. Of particular relevance to diabetes, several miRNA families demonstrably impact both insulin signal transduction and glucose metabolism [7, 8]. This accumulating evidence improve the miRNA-29 can represent a novel therapeutic target for T2DM management, particularly in cases with concurrent HSV-1 infection. However, substantial research gaps remain regarding the precise molecular mechanisms involved. Further studies should prioritize: elucidating the causal relationships between chronic HSV-1 infection and miR-29 dysregulation, and developing targeted interventions that modulate miRNA activity in diabetic populations with evidence of herpesvirus infection.

2. Materials and Methods

2.1. Sample Collection and Preparation

A case-control study based on two groups; the first consisted of 89 ill persons who previously diagnosed with Type 2 Diabetes mellitus by the physician at the Diabetes Center in Marjan Medical City, Babylon province, this group included (45 males and 44 females) with age ranged (40 – 70) years, compared to the second group that composed of 88 healthy individuals (44 males and 44 females), with age ranged (40 – 70) years, The participants of this group were selected from persons without T2DM symptoms, and without history of autoimmune illnesses. T2DM Patients' groups were diagnosed in accordance to the laboratory findings as well as clinical examinations were made by the physicians at the Diabetes Center in Marjan Medical City, Babylon province. A volume of venous blood samples (5 ml) was gathered from the participants of T2DM and the healthy groups from December 2024 – March 2025 and divided into two partitions, 2ml of collected blood were put in an EDTA tube (AFCO, Jordan) mixing with triazole (TransGen, China), and frozen at -80° C until the evaluation of other understudying parameters were done, which included miRNA-29a gene expression by Real time PCR (Analytika Jena, Germany), the second partition of the collected blood (3ml) was put in a gel tube (Orsin, China), and left to clot then separated using a centrifuge (4500 rpm for 10 min) to get the serum that used in the assessment of anti-HSV-1 IgG antibody and TNF-a serum level (bioassay technology laboratory (BT.LAB), China) by using ELISA technique.

2.2. Study Design

The study design included measurement of miRNA-29a level by using the real-time PCR technique, the assessment of anti-HSV-1 IgG antibody, also TNF-α serum level by using the ELISA technique.

2.3. Data Collection

Medical data is collected by a short structured questionnaire, which includes information on sex, age, the period of T2DM, other chronic diseases, living location, lifestyle, and other additional data which recorded on a "data collection sheet", designed for this study.

2.4. Ethical Committee

The participants were notified about the research aim, besides a verbal agreement has been taken from each participant. In addition, its protocol was approved by the University of Baghdad, College of Science Ethical committee (Ref: CSEC/0125/0001).

Table 1.
miRNA primers and probes sequence.

miRNA-29a RT primer	**Sequence 5` to 3`**
miRNA-29a Forward primer	ACACTCCAGCTGGGTTTGGAGTCT
miRNA-29a reverse primer	CTCAACTGGTGTCGTGGA
RNU6 forward primer	CTCGCTTCGGCAGCACAT
RNU6 reverse primer	TTTGCGTGTCATCCTTGCG

Source: Wang, et al. [9]

2.5. Molecular Subject

2.5.1. miRNA-29a Gene Expression

The sequences of primers which is employed in real-time PCR for miRNA-29a gene expression detection were reported Wang, et al. [9] appear in Table 1**.** Beside the Rt-qPCR which is used for miRNA-29a gene expression

detection, the housekeeping gene was RNU6 provided by (Macrogen company, Korea), Table 1.

2.6. Total RNA Extraction

Total RNA was isolated and purified from 2 ml freshly whole blood for 80 samples of 177 collected samples (40 patients and 40 control) using TransZol reagent kit according to the manufacturer's protocol (Boditech, Korea).

Primers' preparation

The lyophilized primers from Macrogen Company (Korea) were melted in an appropriate nuclease-free H_2O to reach (100 pmol/µl), which was employed as a stock solution and kept at -20 °C. These primers' final solution was produced from combining 90µl of nuclease-free H_2O with 10µl of primer stock solution, yielding a final primer solution (10pmol/µl).

2.7. Reverse Transcription

The process involved the conversion of total RNA to complementary DNA (cDNA) using the Script Reverse Transcriptase Kit and following the manufacturer's instructions (ELK Biotechnology, Chine). The reaction took place in a volume of 20 µl, the thermal cycler steps for the cDNA reverse transcription process were carried out according to the details provided in Tables 2 and 3.

Table 2.
GoTaq® 1-Step RT-qPCR Reaction Mix.

Component	Final Volume(µı)	Concentration
GoTaq® qPCR Master Mix, 2X	10	1X
GoScript™ RT Mix for 1-Step RT- qPCR (50X)	0.4	1X
Forward Primer	1.5	300 nM
Reverse Primer	1.5	300 nM
RNA template	4	10^2 ng
$MgCl_2$	1.6	≥2mM
Nuclease free water	1	
Final volume	2 x 10 µı	
CXR Reference Dye	0.33 µl	500 nM

Table 3.
One-Step RT-qPCR Programs.

RT-qPCR Programs' steps	Temperature	Duration	Cycle
Reverse transcription	37°C	15 min	1
Reverse transcriptase inactivation and start activation of GoTaq DNA Polymerase.	95 °C	10min	1
Denaturation	95 °C	10sec	45
Annealing	58 °C	30sec	45
Extension	72 °C	30sec	45
Melt Curve	60-95 °C	15sec	1

2.8. Estimation of total RNA purity

The total RNA extracted by the RNAzol kit was checked using a UV/Visible spectrophotometer instrument (Shimadzu, Japan) to determine the RNA concentration and purity at 260/280 nm.

2.9. Reference Gene Selection

RNU-6 was selected as the reference housekeeping gene because it was more consistently expressed in the serum samples. Beside that the appropriate gene source must have a high level of expression, be stable and expressed in every sample, and have a convergent expression level pass the whole samples [10].

2.10. Determination of miR-29a and RNU6 Reference Gene Expression in the Studied Samples by One Step RT-qPCR

After thawing on ice, each of the GoTaq® 1-Step RT-qPCR components, total RNA, primers, also nuclease-free H_2O have been mixed gently.

GoScript Reverse Transcriptase and GoTaq qPCR Master Mix were coupled in the GoTaq 1-Step RT-qPCR System to provide a single-step, real-time amplification response. The system included a unique fluorescent DNA binding dye (Sybr Green Dye, Promega Corporation, USA) and was tuned for RT-qPCR. This method utilizes GoTaq 1-Step RT-qPCR to facilitate RNA expression.

2.11. Data Analysis of RT-qPCR

The gene expression level was calculated according to instructions recorded by Schmittgen and Livak [11]. This was accomplished by computing the Cycle Threshold Ct value of all participant's miRNA gene beside the reference gene. The base gene has obtained for qPCR normalization by deducting the corresponding miRNA's Ct value from the reference gene's (RNU6) Ct value in the manner described below:

$$\Delta Ct = Ct\ (targeted\ miRNA)\ -\ Ct\ (Endogenous\ control\ or\ reference\ gene\ (RNU6),$$

also ΔΔCt value for all participant's miRNAs were counted by:

$$\Delta\Delta Ct\ =\ \Delta Ct\ (treated\ sample)\ -\ \Delta Ct\ untreated\ sample\ (control)$$

To count the Relative Quantification RQ or gene fold (expression) as follow:

$$RQ\ =\ 2-(\Delta\Delta Ct)$$

2.12. Immunological Assay

The ELISA kits of anti-HSV-1 IgG antibody, TNF-α (Bioassay Technology Laboratory, China) were used the qualitative and quantitative measurements respectively in the serum. The process was done according to the manufacturer instructions of anti-HSV-I IgG antibody and TNF-α.

2.13. Statistical Analysis

The mean and standard error for the numerical data are determined using statistical analysis. The independent t-test and ANOVA table are used to determine the likelihood. The frequency and percentage are computed for categorical data, while the probability is computed employing Pearson chi-square, that reached less than 0.05, the likelihood was considered significant.

3. Results and Discussion

The survey involved 89 ill persons (45), 50.56% males, and (44) 49.44% females recently diagnosed with type 2 diabetes mellitus (T2DM), besides 88 healthy individuals (44) 50.0% males and (44) 50.0% females serving as non-diabetic healthy controls. All participants underwent laboratory testing, including fasting blood sugar (FBS) and glycated hemoglobin (HbA1c), additionally the RNA extraction and the assessment of miRNA-29a gene expression, anti-HSV-1 IgG antibody, and TNF-α levels.

In Table 4, the results of age for T2DM patients and control groups are indicated as non-significant variations ($p > 0.05$) in age mean between T2DM ill participants and the healthy control (56.72 ± 8.02 years for T2DM patients and 53.41 ± 9.57 years for healthy individuals). In terms of sex distribution, this article, the sample distribution by sex does not differ statistically significantly about the two groups. The percentage of females was 49.44.% (44) in the T2DM patients group compared with 50.0% (44) in the control group.

Table 4.
Baseline characteristics and healthy status of study participants.

Parameters		Control groups (n= 88)	Patient group (n=89)	Probability
Age mean ± SE (Years)		53.41 ± 9.57	56.72 ± 8.02	P > 0.05
BMI mean ± SE (Kg/m^2)		22.75 ± 0.35	29.82 ± 0.31	P < 0.001
Sex (%)	No. Males	44 (50.0)	45 (50.56)	P > 0.05
	Females	44 (50.0)	44 (49.44)	

Others found that obesity is a considerable risk factor for type 2 diabetes because it increases insulin resistance [12]. Similarly, others reported that central obesity, often measured through BMI, is closely linked to metabolic disorders, including insulin resistance and elevated fasting blood glucose levels [13]. Therefore, the elevated BMI observed among diabetic patients in this study may work a critical part in the progression of T2DM. Effective weight control strategies may represent a crucial preventive approach for reducing the population-level burden of T2DM. This perspective aligns with current epidemiological data demonstrating a concerning shift in disease patterns—what was traditionally considered an adult-onset condition now increasingly manifests in pediatric and adolescent populations [14]. Researchers attribute this trend to the global rise in childhood obesity and sedentary behaviors, coupled with worsening dietary patterns [15]. The cumulative buildup of cellular damage that occurs with aging eventually reduces the ability of tissues and organs to function [16]. Numerous studies have proposed that a decline in functional β-cell mass with age is the cause of β-cell malfunction in the context of type 2 diabetes mellitus (T2DM). However, more previous research proposed that the intrinsic functionality of β-cells remains largely intact, and it is the aging systemic environment – marked by chronic inflammation, oxidative stress, and metabolic dysregulation – that disrupts β-cell performance and insulin secretion [17].

Additionally, others have looked at the correlations between age and the prevalence of type 2 diabetes, with varying degrees of success. However, an Iraqi study found no significant correlation between the age of patients and the prevalence of type 2 diabetes, indicating that other physiological or environmental factors might be more sufficient in the onset of illness Hasan and Salloom [18].

Al-Musawi, et al. [19] demonstrated that inherited genetic markers and epigenetic modifications show stronger associations with T2DM susceptibility than chronological age or biological sex in Middle Eastern cohorts. This conclusion aligns with multinational researchers [20] whose analysis of global health data revealed striking regional variations in diabetes prevalence. The observed epidemiological patterns reveal complex interactions between cultural influences and biological factors. Traditional food preparation methods, region-specific activity levels, and lifestyle customs appear to modify genetic risk through multiple biological pathways. These relations create disease risk profiles that cannot be described by basic demographic classifications alone.

Patient age at diagnosis nevertheless maintains significant clinical relevance. The Australian Diabetes Cohort Study, identified particularly concerning results for early-onset cases, which patients diagnosed before age 40 faced substantially

greater risks (2.3 times higher) for kidney failure, vision loss, and nerve damage compared to those developing diabetes later in life. Numerous international studies have supported this concerning trend, proposing that prolonged exposure to fluctuating blood glucose levels and metabolic imbalances over the course of life may contribute to cumulative harm [21].

Although these patterns have been widely observed, it remains unclear whether age and sex independently contribute to disease risk or if they are secondary to other interacting physiological or environmental factors. In poor and up- poor line countries, including Iraq, access to healthcare, public health education, and early screening may affect diagnosis rates and outcomes more than demographic variables themselves [22].

The authors illustrated no considerable sex-based differences in the prevalence of T2DM among the participants, which agrees with previous literature suggesting that males and females exhibit similar rates of T2DM when evaluated biologically. Shrestha and others [23] reported comparable diabetes prevalence between males and females, attributing this to parallel mechanisms in insulin sensitivity and secretion across sexes. Furthermore, some of scientific emphasized that while lifestyle and hormonal factors may differ, the physiological response related to insulin resistance and β-cell function remains largely consistent between males and females, supporting the notion of biological similarity in T2DM susceptibility. These findings support the balanced sex's distribution in our study sample, which included 89 T2DM patients (45 males and 44 females) and 88 healthy controls (44 males and 44 females) [24].

However, others have demonstrated that sex shows a critical part in the progression of T2DM. Females generally exhibit greater sensitivity to insulin and a higher predisposition to sympathetic cardio-metabolic risk factors than men, this disparity is largely attributed to sex hormones and their receptors, which significantly influence metabolism, body composition, endothelial function, and chronic inflammation [25]**.** Estrogen was favorable metabolic outcomes in females by modulating fat distribution and giving protection effects against β-cell apoptosis. Also, estrogen has role in insulin sensitivity [26].

For instance, in 2013, the several of males diagnosed with diabetes was 14 million in comparison with females [24]. However, the disease manifests differently between sexes, women who having the T2DM can be under a considerably dangers of cardiovascular complications, including heart failure, and face a greater mortality risk from such conditions compared to diabetic men [27]. These sex-based variations in incidence also outcomes are thought to be influenced not only by biological mechanisms but also by psychosocial and behavioral factors [24, 28]. Also, the findings in Table 5 and Figure 1 presented two diabetic-related parameters (FBG, HbA1c) in T2DM patients and control sets. A highly considerable increase (P<0.01) was found in the FBG and HbA1c levels in the patients (194.99 ± 9.66 mg/dl) and (8.26 ± 0.247%) respectively as compared with control (56.72 ± 8.02 mg/dl) and (5.40 ± 0.03%) respectively.

Table 5.
Glycemic profile variation in T2DM *vs.* control.

Parameters	Control group	Patients group	Probability
FBG mean ± SE (mg/dl)	95.42 ± 0.96	194.99 ± 9.66	P < 0.001
HbA1C mean ± SE (%)	5.40 ± 0.03	8.26 ± 0.247	P < 0.001

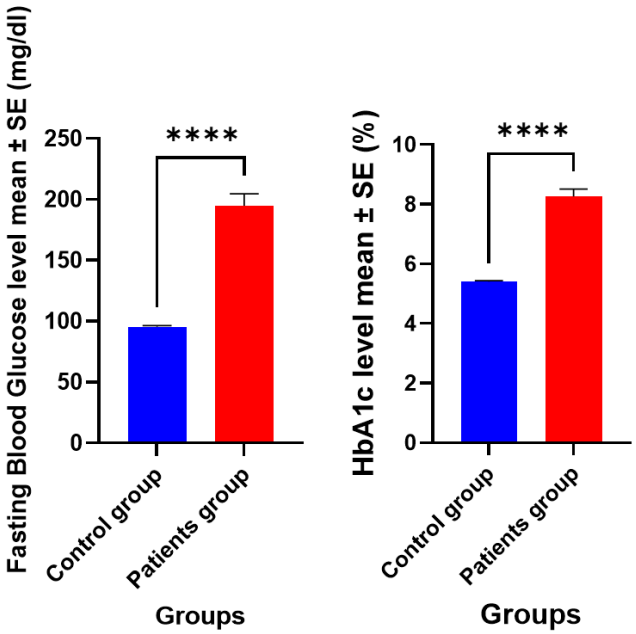

Figure 1.
FBG and HbA1C levels in the studied groups.

The correlation of the fasting blood glucose (FBG) beside glycated hemoglobin (HbA1c) is crucial in managing type 2 diabetes. FBG measures glucose concentration after fasting, providing immediate insight into a patient's basal glucose

level, while HbA1c reflects average plasma glucose concentration over 8-12 weeks, indicating long-term glycemic control [29].

Studies show a positive correlation between FBG and HbA1c levels in diabetic patients, crucial for monitoring disease progression and evaluating therapeutic strategies. Consistent rises indicate poor glycemic control, requiring medication, diet, or lifestyle adjustments [29]. Therefore, simultaneous monitoring of FBG and HbA1c enhances the accuracy of diabetes assessment and supports personalized treatment planning. The research, along with a 2022 Iraqi study found elevated levels of FBS and HbA1c in individuals with type 2 diabetes [30].

The study emphasizes the significance of biomarkers in diabetes management, highlighting that high levels of glycated hemoglobin (HbA1c), particularly over 6.5%, indicate impaired metabolic control in diabetes patients, indicating potential T2DM risk [31]. Research established that an HbA1c threshold of 6 mg/dl is a reliable cutoff for distinguishing diabetic and non-diabetic individuals [32]. Others found a significant positive correlation between FBG and HbA1c levels in T2DM patients, indicating that FBG can be a useful surrogate in settings where HbA1c testing is less accessible [33]. Research registred a moderate correlation between FBG and HbA1c and proposed an FBG cutoff point of 130 mg/dL for predicting HbA1c levels of 7% or higher [34]. Others highlighted the varying correlation for each of the FBG and HbA1c across age and ethnic groups, emphasizing the need for personalized diagnostic criteria and the clinical relevance of HbA1c in diabetic patients [35]. Groups of scintific found that FBG and postprandial glucose levels correlate well with HbA1c, but postprandial glucose has a marginally stronger predictive value. Chronic hyperglycemia stresses pancreatic β-cells, leading to overstimulation and excessive insulin secretion. This leads to β-cell dysfunction, glucotoxicity, and insulin resistance, causing T2DM. This resistance impairs glucose uptake, causing elevated extracellular glucose concentrations and further hyperglycemia [36]. Glucotoxicity is a condition causing impaired insulin gene expression, reduced insulin secretion, and increased β-cell apoptosis related to oxidative stress from elevated glucose levels [35]. As β-cell function deteriorates, insulin resistance develops, especially in peripheral tissues as skeletal muscle.

Researchers investigated the expression levels of miRNA-29a in T2DM ill person's in comparison with healthy groups. They conclusions revealed a significant upregulation of miRNA-29a in T2DM patients, suggesting a potential role of miRNA-29a in the early stages of T2DM pathogenesis. The expression levels of miRNA-29a were quantitatively analyzed in both control and patient groups using RT-qPCR. As illustrated in Table 6, a significant elevated fold change of miRNA-29a in T2DM patients (14.51 ± 0.74) relative to controls (1.02 ± 0.03) ($P < 0.001$), (Figure 2).

Table 6.
miR-29a expression level in T2DM *vs.* control.

Groups	Mean ± SE				
	RNU6 ct	**miR-29a ct**	**miR-29a Δct**	**miR-29a ΔΔct**	**miR-29a folding**
Control	19.05 ± 0.39	26.98 ± 0.41	7.93 ± 0.04	0.012 ± 0.039	1.02 ± 0.03
Patients	19.63 ± 0.42	23.75 ± 0.40	4.12 ± 0.07	-3.80 ± 0.069	14.51 ± 0.74
Probability	P > 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001

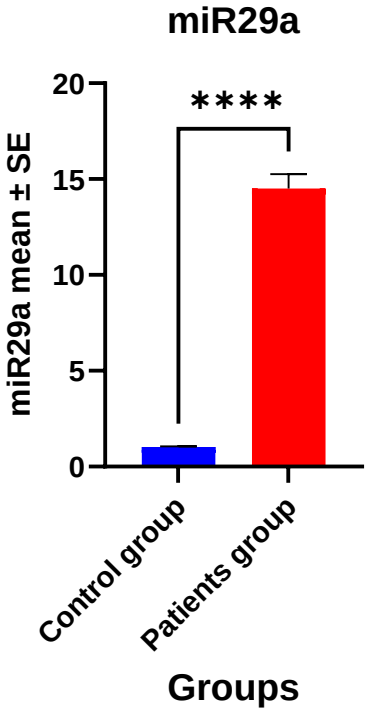

Figure 2.
miRNA-29a level (fold) in T2DM group compared to control group.

The status of anti-HSV-1 IgG antibody seroprevalence and TNF-α levels were measured for diabetic patients and healthy individuals. The presence of anti-HSV-1 IgG antibodies was evaluated in both the control and type 2 diabetes mellitus (T2DM) groups, Table 7 and Figure 3, 4. Only one individual (1.12%) of the patient group tested positive for anti-HSV-1 IgG antibody compared to 0.0% seropositive in the control group. The vast majority of participants in both groups were negative for HSV-1 IgG, with 100.0% of the controls and 98.88% of the patients testing negative. The statistical analysis revealed no significant difference in HSV-1 IgG seropositivity between the two groups ($P > 0.05$), indicating that prior HSV-1 exposure, as measured by IgG antibodies, was not considerably associated with T2DM in this sample. Furthermore, the TNF-α revealed a statistically significantly higher serum level in T2DM ill people in comparison to the control. The mean of TNF-α level of the patient set was 131.04 ± 7.99 pg/mL, compared to 77.53 ± 3.79 pg/mL in the control group, with a statistically considerable variation ($P < 0.0001$), as shown in Table 7 and Figure 4. The increasing level of TNF-α in T2DM ill persons in comparison with the control can have a part in the growth and pathogenesis of T2DM [37].

Table 7.
The seroprevalence of anti-HSV-1 IgG antibody status and TNF-α level in the studied groups.

Studied groups	Anti-HSV-1 IgG antibody status		TNF-α level mean ± SE (pg/ml)
	Positive	Negative	
Control group	0 (0.0)	88 (100.0)	77.53 ± 3.79
Patients group	1 (1.12)	88 (98.88)	131.04 ± 7.99
Probability	P > 0.05		P < 0.0001

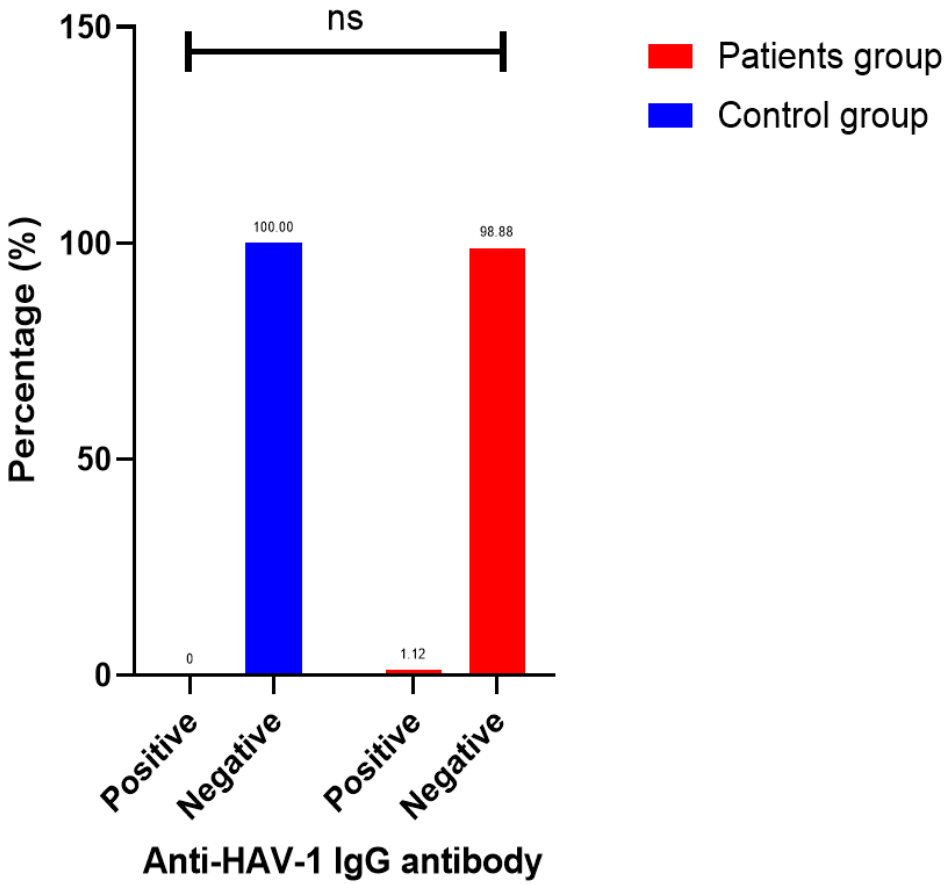

Figure 3.
The seroprevalence of anti-HSV-1 IgG antibody status in the studied groups.

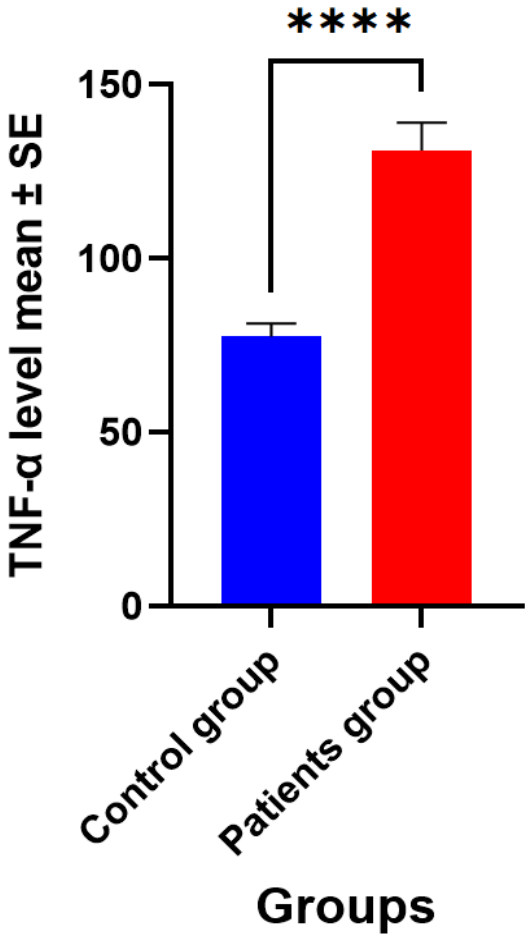

Figure 4.
TNF-α level in T2DM vs. control.

The study improved that have no considerably link between HSV-1 IgG seropositivity and type 2 diabetes mellitus, despite previous hypotheses suggesting a link between chronic herpesvirus infections and metabolic disorders. The low prevalence of HSV-1 IgG antibodies in sick persons and controls suggests past infection evidence may not be a reliable biomarker for T2DM risk. The multifactorial nature of T2DM, influenced by genetic, lifestyle, and environmental factors, necessitates further research involving diverse populations and viral load and inflammatory markers.

The study aligns with conclusions finding that persistent HSV-1 infection doesn't predict diabetes incidence, despite adjusting for obesity, age, and inflammatory markers [38]. They found no considerable variations in HSV-1 IgG levels about each of the diabetic and non-diabetic groups.The study aligns with the Multi-Ethnic Study of Atherosclerosis, which found no significant correlation between HSV-1 seropositivity and T2DM development after adjusting for demographic variables , also found no considerably association between serum anti-HSV-1 IgG antibody levels and T2DM incidence, suggesting that HSV-1 IgG seropositivity may not be a reliable biomarker for T2DM risk [39].

Previous studies suggest a possible link between chronic viral infections (HSV-1) and the development or exacerbation of type 2 diabetes, suggesting that virus-induced inflammation could contribute to insulin resistance [40]. Studies on HSV-1 infection and Type 2 Diabetes differ due to design, demographics, sample sizes, and methodologies, and cross-sectional nature limits causal association. The study's low anti-HSV-1 IgG antibody positivity may be due to geographic or demographic factors, such as immune system differences. It's crucial to note that HSV-1 IgG only indicates past infection, not reactivation status or viral load, which may impact metabolic or inflammatory pathways. TNF-α, a pro-inflammatory cytokine, is involved in the pathogenesis of various diseases and insulin resistance in obesity and type 2 diabetes. Its levels can increase systemic insulin resistance in human fat, and its expression is higher in T2DM patients' muscles than non-DM patients [37].

Other hands, there is who discover elevated TNF-α levels in individuals with type 2 diabetes (T2DM), which were linked to insulin resistance and HbA1c levels, suggesting a pathogenic role in metabolic dysfunction, particularly in obese diabetics, and impaired glucose uptake. Yao, et al. [26] study found no considerable variations in TNF-α levels between diabetic ill persons and controls, possibly due to variations in patient populations, disease duration, BMI, and therapeutic interventions. MiRNAs, small RNAs 18-25 nucleotides long, regulate T2DM pathophysiology by inhibiting or degrading mRNA targets, playing a critical role in RNA synthesis [2]. MiRNA-29a, a biomarker and potential therapeutic target, has been linked to insulin signaling, glucose metabolism, fibrosis, inflammation, and diabetic complications, with its expression patterns varying depending on disease stage and tissue type [41, 42].

The study found a significant increase in miRNA-29a in T2DM patients, consistent with previous studies linking miRNA-29a dysregulation with metabolic dysfunction [3, 41]. Studies show miRNA-29a's role in glucose metabolism and insulin signaling pathways, its correlation with impaired insulin secretion and β-cell dysfunction, and its potential role as an early biomarker for Type 2 Diabetes. A Nepali study found high miR-29a levels in T2DM patients, indicating potential use as a non-invasive biomarker for early detection and prediction of the condition [42].

Figure 5 presents the Pearson's correlation coefficients among the studied variables in T2DM patients, including the age, BMI, FBG, HbA1c, TNF-α, and miRNA-29a. Findings of the correlation analysis showed a significantly positive correlation between BMI with FBG ($r = 0.409$, $p < 0.01$), suggesting that an increase in body mass index is correlated with increased fasting blood glucose. A similar considerable positive correlation was observed between BMI and HbA1c ($r = 0.421$, $p < 0.01$), implying a potential link between obesity and poor glycemic control.

A considerable positive correlation was found between BMI and miRNA-29a ($r = 0.649$, $p < 0.01$), indicating a potential regulatory or mechanistic interaction between adiposity and miRNA expression. For FBG, a very strong positive correlation was exhibited with HbA1c ($r = 0.882$, $p < 0.01$). This is expected, as both are key markers of glycemic status and are physiologically related. Also, FBG showed moderately significant correlations with TNF-α ($r = 0.307$, $p < 0.01$) and miRNA-29a ($r = 0.641$, $p < 0.01$), suggesting involvement of inflammatory and molecular pathways in glucose dysregulation. Furthermore, HbA1c was considerable relation with TNF-α ($r = 0.262$, $p < 0.01$), reflecting a possible association between systemic inflammation and chronic hyperglycemia, and with MiR-29a ($r = 0.636$, $p < 0.01$) indicating that miR-29a expression may be upregulated in states of poor glycemic control.

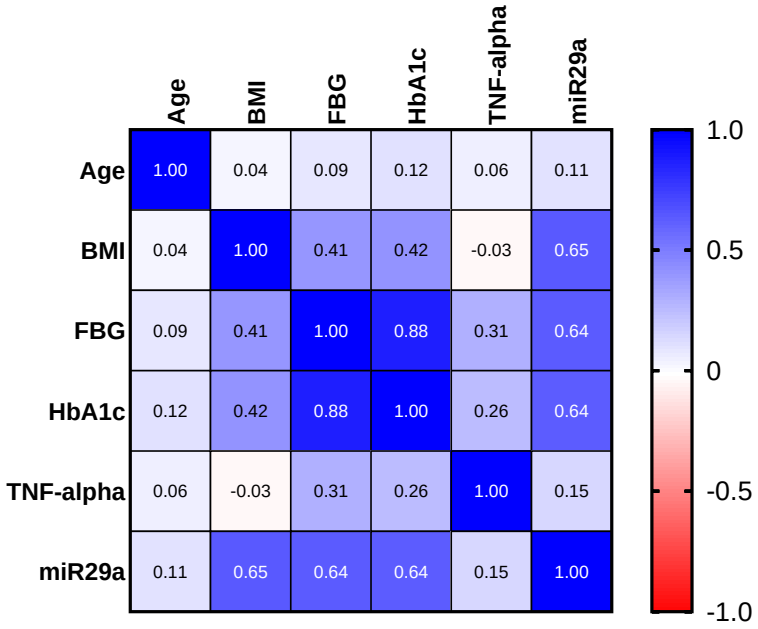

Figure 5.
The Correlations among various studied parameters with T2DM.

Lastly, there was no discernible relationship between miRNA-29a and TNF-α ($r = 0.154$), which may reflect complex or non-linear relationships not captured by Pearson analysis.

The significant positive correlations between miRNA-29a and both FBG and HbA1c in this study suggest that miRNA-29a may be actively involved in glucose dysregulation and chronic hyperglycemia in T2DM. This aligns with findings by Sun, et al. [43] who reported promoted levels of circulating miRNA-29a in T2DM patients and its role in impairing insulin signaling via downregulation of Insulin Receptor Substrate-1 (IRS-1). Similarly, others demonstrated that miRNA-29a negatively regulates glucose transporter Glucose Transporter Type 4 (GLUT4) in adipocytes, thus contributing to insulin resistance and hyperglycemia. These studies support the current findings that increased miRNA-29a levels correlate with poor glycemic indicators [44].

The observed positive correlation between BMI and miRNA-29a ($r = 0.649$, $p < 0.01$) suggests a potential link between miRNA-29a expression and adiposity. This is consistent with results by Massart, et al. [8] who showed that obese individuals with T2DM had higher levels of miRNA-29a compared to non-obese diabetic patients. This correlation supports the hypothesis that miRNA-29a may act as a mediator between obesity-induced inflammation and insulin resistance. This supports the notion that miRNA-29a is involved in adipose tissue inflammation and metabolic dysfunction. Previous studies have demonstrated that miRNA-29a is upregulated in obese individuals and contributes to insulin resistance via targeting insulin signaling pathways [43].

The extremely high correlation between FBG and HbA1c ($r = 0.882$, $p < 0.01$) validates the expected relationship between short-term and long-term glycemic markers. The BMI's correlation with both glycemic markers suggests that obesity remains a significant contributor to poor glycemic control. Overall, these findings suggest that miRNA-29a plays a crucial role in the metabolic and inflammatory pathways underlying T2DM, particularly in relation to glycemic control and

obesity. The consistency of these results with previous studies highlights its potential as a biomarker or therapeutic target in T2DM Several studies have implicated miRNA-29a in the dysregulation of metabolic and inflammatory pathways associated with T2DM. Others reported that miRNA-29a impairs insulin signaling through inhibition of the phosphoinositide 3-kinase/ protein kinase B (PI3K/AkB) pathway [41, 42]. Reserachers highlighted its involvement in modulating immune responses by regulating pro-inflammatory gene expression [2] while others demonstrated that miRNA-29a negatively affects glucose metabolism by targeting SIRT1 in skeletal muscle cells. These findings suggest that miR-29a plays a pivotal role in the interplay between insulin resistance, inflammation, and glucose homeostasis,

Although miRNA-29a showed no correlation with TNF-α ($r = 0.154$, $p > 0.05$), this may indicate a weaker or more indirect interaction. This is consistent with results by Afsharmanesh, et al. [41] who demonstrated that TNF-α can downregulate the expression of miRNA-29a through the activation of the nuclear factor-kappa B (NF-κB) signaling pathway in various cell types.

Figure 6 represented a receiver operating characteristic (ROC) curve; the curve demonstrated a perfect classification performance, with Area under the Curve (AUC) of 1.0. this suggests the model effectively distinguishes between positive and negative cases. However, while a high sensitivity (1.0) is ideal in disease screening, the very low specificity (0.023) implied that many false positives are present. Such results imply that the model correctly identifies all actual positive cases, making it highly reliable for screening purpose. However, its low specificity means that many healthy individuals are misclassified as diseases, which could lead to unnecessary interventions or further testing. Balancing sensitivity and specificity is crucial in clinical applications, where both minimizing false positives and maximizing true positives are essential for accurate diagnosis.

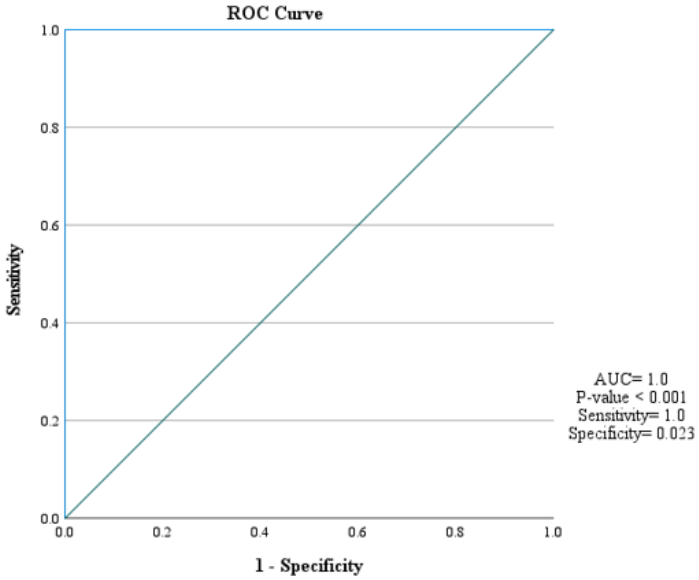

Figure 6.
ROC analysis of miR-29a.

Previous studies explored the significance of miR-29a in T2DM using the ROC analysis, aligning with the current study, suggested a significantly elevated serum miR29a level in T2DM, and ROC analysis confirmed its potential as a biomarker for metabolic disorders [40]. Also, study emphasized on the levels of miRNA-29a in T2DM group, demonstrating that elevated miRNA-29a expression correlate with disease progression [43]. Similarly, study investigated the expression of miRNA-29a in the newly diagnosed and treated T2DM patients, revealing that miRNA-29a levels were significantly higher in diabetic individuals, and miRNA-29a has a strong diagnostic potential for distinguishing T2DM cases and assessing β-cell dysfunction [40, 43].

4. Conclusion

The study highlights the role of miRNA-29a in T2DM development, as its marked elevation was related to both glycemic indicators and inflammatory status. Additionally, increased TNF-α levels reinforce the contribution of inflammatory mechanisms to the diseases' progression. However, the absence of HSV-1 IgG seropositivity and T2DM association indicates that viral exposure alone may not be a determining factor in disease development. The ROC analysis highlighted the need for refining diagnostic model to improve specificity while maintaining sensitivity. Future research should focus on validating miRNA-29a as a biomarker, investigating its mechanistic character in insulin resistance, and exploring potential therapeutic interventions targeting miRNA regulation in diabetic patients.

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