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Non-Coding RNAs and Their Associated Genes in Diabetic Foot Ulcer and Wound Healing Process

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ABSTRACT

Diabetic foot ulcer (DFU), a devastating complication of type 2 diabetes mellitus (T2DM), is a major contributor to non-traumatic lower limb amputations globally. Despite advances in clinical care, the molecular mechanisms underlying delayed wound healing in DFU remain hopelessly exposed. This study investigates the regulatory roles of small (micro RNAs) specifically, miR-143, miR-145, and miR-146a and long non-coding RNAs (lncRNAs): GAS5 and WAKMAR2 and their downstream gene targets *MAP3K7, LOX, THBD, STAT1*, and *NFκB* in DFU pathogenesis. A total of 150 subjects were enrolled, comprising 50 DFU patients, 50 T2DM patients without complications, and 50 healthy controls. Quantitative real-time PCR (qRT-PCR) was utilized to quantify differential expression of selected miRNAs, lncRNAs, and their corresponding target genes in tissue samples. The results demonstrated a consistent downregulation of miR-143, miR-145, and miR-146a in DFU patients compared to T2DM and healthy groups, while their target genes *MAP3K7, LOX,* and *THBD* exhibited significant upregulation ($P < 0.01$). Similarly, WAKMAR2 was markedly downregulated in DFU tissues, whereas GAS5 was upregulated. This dysregulation was reflected in their target gene expression, with *NFκB* upregulated and *STAT1* downregulated in DFU samples. These findings suggest that aberrant expression of specific non-coding RNAs contributes to the disruption of key signaling pathways involved in inflammation, proliferation, and tissue remodeling, hallmark phases of wound healing. Collectively, the altered expression profiles of these ncRNAs and their gene targets may serve as novel diagnostic biomarkers and therapeutic targets for early intervention and improved management of DFU.

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Key words
Diabetic foot ulcer, micro RNAs, Long noncoding RNAs, T_2DM, Angio genic growth factor, Wound healing

INTRODUCTION

Diabetes mellitus (DM), particularly type 2 diabetes (T2DM), is a complex metabolic disorder characterized by chronic hyperglycemia resulting from insulin resistance and pancreatic β-cell impairment (Vikraman *et al*., 2024). Lifestyle factors such as high caloric intake and sedentary behavior have led to a dramatic increase in global T2DM prevalence, with profound implications for public health (Aghayants *et al*., 2024; Rasmi *et al*., 2023). Among the serious microvascular complications of T2DM, diabetic foot ulcer (DFU) represents a leading cause of morbidity. Approximately 15 % of diabetic individuals develop DFUs during their lifespan, and an alarming 84 % of these cases eventually require limb amputation (Akhtar *et al*., 2022). This phenomenon is equally observed in South Asian populations, including Pakistan, where limited healthcare resources further exacerbate the clinical burden (Hussain *et al*., 2022).

DFU pathogenesis is driven by the intersection of peripheral neuropathy, peripheral arterial disease (PAD), and chronic inflammation (Patel *et al*., 2019). Neuropathy impairs nociceptive feedback, leading to unnoticed foot injuries, while PAD-induced ischemia limits perfusion and oxygen delivery (Marco *et al*., 2021; Mohsin *et al*., 2024). Additionally, hyperglycemia is associated with immune dysfunction, leukocyte chemotaxis and oxidative burst are impaired, resulting in persistent infection and inflammation (Li *et al*., 2023). At the molecular level, DFU microenvironments exhibit downregulated angiogenic growth factors such as VEGF, PDGF, and IGFs, due to hyperglycemia-induced oxidative stress, the accumulation

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of advanced glycation end products (AGEs), and epigenetic dysregulation (Yang *et al*., 2024). Simultaneously, pro-inflammatory cytokines (IL-6, IL-8, TNF-α), matrix-degrading enzymes (MMP-9), and transcription factors (NF-κB, c-Myc) are overexpressed, resulting in futile fibroblast proliferation, delayed re-epithelialization, and extracellular matrix (ECM) breakdown (Ozdemir and Feinberg, 2019; Huang *et al*., 2025; Tang and Ran, 2018).

Emerging evidence highlights the role of non-coding RNAs (ncRNAs) including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) in orchestrating molecular pathways integral to wound healing. These molecules modulate gene expression at transcriptional and post-transcriptional levels and critically influence inflammation, angiogenesis, cell migration, and remodeling.

ncRNAs including miRNAs and lncRNAs, exert a direct and causative influence on the development and progression of DFU by modulating critical gene expression networks that govern wound healing (Vikraman *et al*., 2024; Aghayants *et al*., 2024; Rasmi *et al*., 2023). These ncRNAs regulate the transcription and translation of specific target genes involved in inflammation, immune response, angiogenesis, and tissue remodeling all essential processes for effective wound repair. For instance, miR-146a directly suppresses key components of the NF-κB signaling pathway, such as IRAK1 and TRAF6, thereby modulating the inflammatory response; its downregulation in DFU leads to unchecked inflammation and impaired healing (Vikraman *et al*., 2024; Rasmi *et al*., 2023). Similarly, the miR-143/145 cluster targets MAP3K7, LOX, and THBD, genes associated with ECM integrity and vascular homeostasis, and their dysregulation contributes to chronic wound pathology (Aghayants *et al*., 2024). On the other hand, lncRNAs such as GAS5 and WAKMAR2 epigenetically control transcription factors like STAT1 and NF-κB, respectively, influencing macrophage polarization and keratinocyte migration (Ozdemir and Feinberg, 2019). Altered expression of these lncRNAs disrupts the tightly regulated transition between the inflammatory and proliferative phases of healing, leading to delayed or non-healing ulcers. Together, the aberrant expression of ncRNAs and their downstream gene targets forms a direct molecular axis that actively drives the pathophysiological mechanisms of DFU, rather than being a mere consequence of chronic hyperglycemia or secary tissue damage. Thus, targeting these ncRNA gene regulatory networks offers a compelling avenue for early diagnosis, risk stratification, and therapeutic intervention in diabetic foot ulcer management (Yang *et al*., 2023).

The regulatory relationship between miR-143 and its target gene MAP3K7 (Mitogen-activated protein 3 Kinase 7) plays a critical role in modulating inflammatory and stress response pathways, particularly within the context of DFU pathogenesis. miR-143, commonly expressed in vascular smooth muscle and epithelial cells, acts as a negative regulator of MAP3K7, a central component of the TAK1-NF-κB signaling axis. In normal physiological conditions, miR-143 maintains homeostasis by suppressing MAP3K7 translation, thereby attenuating downstream pro-inflammatory cytokine expression (Rasmi *et al*., 2023). However, in DFU patients, downregulation of miR-143 leads to overexpression of MAP3K7, resulting in heightened activation of NF-κB, increased transcription of TNF-α and IL-6, and a sustained pro-inflammatory environment that severely impairs wound healing (Laczko *et al*., 2020). This dysregulation not only prolongs the inflammatory phase of wound repair but also contributes to excessive extracellular matrix degradation and apoptosis of keratinocytes and fibroblasts hallmarks of chronic, non-healing DFU lesions. Experimental studies using qRT-PCR and luciferase reporter assays have confirmed the direct post-transcriptional targeting of MAP3K7 by miR-143, validating this interaction in both diabetic mouse models and human wound tissue samples (Song *et al*., 2025). Thus, the miR-143–MAP3K7 axis serves as a crucial molecular switch whose imbalance is directly implicated in DFU progression, and its restoration may provide a targeted therapeutic approach for modulating chronic inflammation in diabetic wounds.

The lncRNA GAS5 (Growth Arrest-Specific 5) and STAT1 (signal transducer and activator of transcription 1) axis plays a pivotal role in regulating macrophage polarization, inflammatory signaling, and keratinocyte behavior, all of which are crucial for orchestrating wound healing. GAS5 is a lncRNA known for its function as a transcriptional decoy or competitive endogenous RNA (ceRNA) that binds to and regulates transcription factors and microRNAs involved in immune and growth-related pathways. In chronic wounds such as DFUs, GAS5 is consistently upregulated, particularly in macrophage populations and keratinocytes, where it acts as a sponge for miRNAs that normally repress STAT1, thereby promoting the overexpression of STAT1 (Shojaeian, 2024). STAT1 is a transcription factor involved in type I interferon and inflammatory cytokine signaling; its persistent activation in wounds leads to prolonged M1 macrophage polarization, excessive IL-6 and TNF-α secretion, and impaired transition to the proliferative phase of healing (Yu *et al*., 2022). In normal wound healing, downregulation of GAS5 facilitates the resolution of inflammation and supports M2 polarization and tissue remodeling. However, in DFU lesions, GAS5-mediated STAT1 overexpression maintains a pro-inflammatory state, suppresses fibroblast migration,

and inhibits angiogenesis creating a hostile environment for epithelialization and granulation tissue formation (Peng *et al*., 2021). These findings underscore GAS5 as a critical epigenetic regulator in DFU and highlight the GAS5 and STAT1 axis as a promising therapeutic target for modulating chronic inflammation and promoting regenerative healing in diabetic wounds.

The lncRNA WAKMAR2 (Wound and Keratinocyte Migration-Associated lncRNA 2) has emerged as a critical epigenetic regulator of keratinocyte migration and wound re-epithelialization, primarily through its impact on the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway. WAKMAR2 is normally expressed during the early phases of wound healing, where it helps maintain the balance between inflammation and regeneration by repressing hyperactive NF-κB transcriptional activity in keratinocytes and dermal fibroblasts (Xiao *et al*., 2024). In the setting of DFUs, WAKMAR2 expression is significantly downregulated, leading to disinhibition of NF-κB signaling. This results in persistent upregulation of inflammatory cytokines such as IL-6, TNF-α, and IL-1β, sustained recruitment of neutrophils and macrophages, and impaired keratinocyte proliferation and migration (Peng *et al*., 2021). Functionally, WAKMAR2 is known to interact with histone-modifying complexes and transcriptional co-repressors to epigenetically silence NF-κB target genes, such as CXCL1 and ICAM-1, thus modulating leukocyte adhesion and chemotaxis. Loss of this regulatory brake in DFU contributes to a chronic pro-inflammatory environment, increased reactive oxygen species (ROS) production, and MMP-9 activation, which collectively degrade the ECM and delay wound closure (Yang *et al*., 2023). Restoration of WAKMAR2 expression in diabetic wound models has been shown to suppress NF-κB hyperactivation and significantly enhance wound healing rates, positioning the WAKMAR2–NF-κB axis as a therapeutic target for reversing chronic inflammation and improving epithelial regeneration in DFU patients.

The miR-145–LOX regulatory axis has emerged as a crucial epigenetic mechanism involved in the ECM remodeling dysfunction that underpins the chronic nature of DFU. miR-145, which is co-transcribed with miR-143 as part of a conserved bicistronic cluster, plays a critical role in controlling cellular differentiation, migration, and ECM homeostasis in epithelial and vascular tissues. One of the key validated targets of miR-145 is Lysyl Oxidase (LOX), a copper-dependent amine oxidase responsible for catalyzing the covalent cross-linking of collagen and elastin fibers during matrix remodeling (Song *et al*., 2025). Under normal wound-healing conditions, miR-145 helps regulate ECM rigidity by limiting LOX expression, ensuring optimal balance between ECM deposition and degradation. However, in DFU tissues, miR-145 is significantly downregulated, leading to upregulated LOX expression, which in turn causes excessive ECM cross-linking and increased tissue stiffness conditions that impair fibroblast migration, angiogenesis, and re-epithelialization (Laczko *et al*., 2020). This pathological matrix rigidity further contributes to the formation of a fibrotic and non-compliant wound bed, which becomes resistant to closure. Additionally, LOX over activity has been associated with increased oxidative stress and fibrotic signaling through TGF-β1/Smad and Akt pathways, exacerbating wound chronicity in diabetic patients (Yu *et al*., 2022). Experimental inhibition of LOX or restoration of miR-145 expression in diabetic models has been shown to partially reverse these effects, indicating the potential of targeting this axis for therapeutic benefit in chronic wound care.

The miR-146a–THBD (Thrombomodulin) regulatory axis represents a vital molecular pathway that governs both inflammation resolution and vascular integrity during the wound-healing process, and its disruption is critically implicated in the pathology of diabetic foot ulcers (DFUs). miR-146a functions as a negative regulator of inflammatory signaling by targeting key components of the Toll-like receptor (TLR) and NF-κB pathways, such as IRAK1 and TRAF6, thereby attenuating pro-inflammatory cytokine production and promoting tissue repair (Vikraman *et al*., 2024). Recent studies have identified THBD as an additional direct target of miR-146a. THBD encodes thrombomodulin, an endothelial glycoprotein that plays a dual role in anti-coagulation and anti-inflammation, primarily by activating protein C and downregulating thrombin activity (Xiao *et al*., 2024). In DFU patients, miR-146a expression is significantly reduced, leading to the upregulation of THBD and an aberrant pro-thrombotic and pro-inflammatory vascular microenvironment. While THBD expression is normally protective in endothelial biology, its uncontrolled upregulation in DFU has been associated with endothelial dysfunction, impaired leukocyte trafficking, and delayed wound revascularization, ultimately contributing to the chronicity of the wound (Peng *et al*., 2021). Moreover, dysregulated THBD has been implicated in disturbing the VEGF-mediated angiogenic signaling necessary for tissue regeneration. Thus, the miR-146a–THBD axis represents a finely balanced checkpoint in wound inflammation and resolution, and its disruption in DFU pathogenesis highlights both molecules as promising targets for molecular diagnostics and RNA-based therapeutic interventions aimed at enhancing healing and preventing amputation in diabetic patients.

miR-146a mitigates inflammation by targeting

TRAF6 and IRAK1 within the NF-κB pathway. A 2024 study by Vikraman *et al*. (2024) reported reduced miR-146a in Grade 2 to 3 DFUs, correlating with elevated TRAF6, IRAK1, ER stress, and oxidative markers. miR-143/145 cluster regulates vascular smooth muscle cells and insulin sensitivity. It modulates ACE and Akt signaling key pathways in angiogenesis and matrix remodeling and its dysfunction is directly linked to DFU pathogenesis (Song *et al*., 2025).

Other miRNAs miR-16, miR-21, and miR-99a are implicated in inflammatory modulation (via COX-2), apoptosis, and Akt/mTOR signaling, respectively, further influencing reparative mechanisms (Jankauskas *et al*., 2021). Additional miRNAs such as miR-185-5p, miR-132, and miR-221-3p have been shown to attenuate NF-κB signaling, promote M2 macrophage polarization, and enhance angiogenesis emphasizing the complex regulatory milieu within DFU tissue (Yu *et al*., 2022). GAS5 modulates fibroblast proliferation and macrophage polarity via the STAT1 axis. Its upregulation in diabetic wounds promotes M1 polarization, delaying healing. However, GAS5 overexpression can sometimes stimulate lymphangiogenesis, highlighting context-dependent effects (Hu *et al*., 2020).

WAKMAR2 supports keratinocyte migration and inflammatory regulation at the re-epithelialization front. Its downregulation in DFUs has been confirmed in 2024 functional screenings (Xiao *et al*., 2024). Additional lncRNAs including H19, ANRIL, and SNHG26 have been linked to fibroblast activation, ECM remodeling, and phase transition in wound healing. Chronic hyperglycemia triggers oxidative stress through ROS formation and AGEs, which are tightly linked with epigenetic alterations-DNA methylation and histone modifications critical to DFU initiation (Li *et al*., 2025). LncRNAs like GAS5 and SNHG26 act through Nrf2, PI3K/Akt, and HIF-1 signaling pathways to alleviate oxidative stress and improve vascular and immune cell function. A comprehensive 2024 review elucidates how oxidative-stress related lncRNAs influence all stages of diabetic wound healing, presenting new avenues for targeted therapies (Yang *et al*., 2023).

The regulatory landscape of DFU is defined by ncRNA and target gene interactions: MAP3K7 (TAK1) is modulated by miR-143/145 and miR-146a, influencing inflammatory responses and stress signaling (Huang *et al*., 2021). LOX affects collagen cross-linking and matrix stiffening, regulated by these miRNAs. THBD impacts endothelial integrity and coagulation all linked to miRNA dysfunction. STAT1 and NF-κB, major downstream targets of GAS5 and WAKMAR2, coordinate apoptosis and immune pathways vital to wound resolution (Shojaeian, 2024). Despite increasing studies on DFU-related ncRNAs, analyses that integrate miRNA and lncRNA expression with downstream gene profiling in South Asian cohorts remain scarce. Given the unique epigenetic and environmental context of Pakistani populations, this study aims to: Measure expression of miR-143, miR-145, miR-146a, and lncRNAs GAS5, WAKMAR2 in tissue biopsies across three groups: DFU patients, T2DM patients without ulcers, and healthy subjects. Assess levels of key gene targets: MAP3K7, LOX, THBD, STAT1, NF-κB to elucidate regulatory pathways.

Analyze interrelationships between ncRNAs and target gene expression to reconstruct a DFU-specific regulatory network. By investigating both short and long ncRNAs and their molecular targets, this study seeks to: (i) illuminate regulatory mechanisms underpinning impaired wound healing in DFU. (ii) identify novel biomarkers (e.g., underexpressed miRNAs/lncRNAs or upregulated targets) for early DFU detection. (iii) suggest therapeutic targets suitable for ncRNA-based interventions. Understanding ncRNA-driven pathogenesis of DFU in a population-specific framework may contribute meaningfully to personalized medicine and pave the way for innovative, targeted treatments to reduce morbidity and improve patient outcomes worldwide.

MATERIALS AND METHODS

A cross-sectional, comparative molecular study was designed to evaluate the expression of specific non-coding RNAs (miRNAs and lncRNAs) and their target genes in DFU patients, diabetes patients without complications (T2DM group), and healthy diabetic controls.

Individuals aged 50–70 years, diagnosed with Type 2 diabetes mellitus (T2DM) for >5 years, receiving insulin therapy and No history of cancer, autoimmune disorders, or co-infections were included in the study. Subject below 50 and above 70 years of age, type 1 diabetes, on medicine and individuals with other diabetic complications such as nephropathy, retinopathy, or cardiovascular disease were excluded from this study.

Participants (n= 150) were divided into three groups each containing 50 per subject: Group I (Controls) included diabetic patients without any complications, Group II (T2DM) were diabetic patients without DFU and Group III (DFU) were diabetic patients clinically diagnosed with diabetic foot ulcers. Skin biopsy specimens were obtained from the wound edges of DFU patients using sterile punch biopsy techniques (3–4 mm diameter), preserved in RNAlater (Thermo Fisher) and stored at −80°C.

Biochemical testing was performed using spectrophotometric methods with standard clinical diagnostic kits.

RNA isolation

Total RNA was extracted from tissue samples using the following commercial kits: Norgen RNA Purification Kit (Norgen Biotek Corp., Canada), QIAGEN RNeasy Mini Kit (QIAGEN, Germany). The RNA extraction protocol was followed according to each manufacturer's instructions, ensuring the inclusion of on-column DNase I digestion to eliminate genomic DNA contamination.

After extraction, RNA concentration and purity were assessed using the NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, USA) by measuring A260/280 and A260/230 ratios. Acceptable purity was defined as A260/280 between 1.8–2.1 and A260/230 ≥ 1.8.

To validate RNA integrity, a 1.5% agarose gel electrophoresis was performed. Selected RNA samples were further evaluated using an Agilent 2100 Bioanalyzer (optional), and only samples with RNA Integrity Number (RIN) >7.0 were processed for downstream applications. For mRNA enrichment, poly-A RNA selection was conducted using the Lexogen Poly(A) RNA Selection Kit v1.5 (Austria), as appropriate.

CDNA synthesis

Reverse transcription

mRNAs and lncRNAs were converted to cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) and the QIAGEN QuantiTect Reverse Transcription Kit, using oligo(dT) and random hexamer primers. miRNAs were reverse transcribed using a stem-loop RT primer-based method or commercially available miRNA cDNA Synthesis Kit, optimized for small RNA molecules. All reactions were carried out in 20 µL total reaction volume, incubated at 42°C for 60 min followed by enzyme inactivation at 70°C for 10 min.

Expression analysis

Gene-specific primers for miRNAs (miR-143, miR-145, miR-146a), lncRNAs (GAS5, WAKMAR2), and their target genes (MAP3K7, LOX, THBD, STAT1, NF-κB) were designed using the PrimerQuest Tool (Integrated DNA Technologies, IDT, USA) and validated for specificity using BLAST (NCBI). Primer efficiency and specificity were assessed using standard curves and melt curve analysis, respectively.

Expression analysis was performed using SYBR Green-based real-time PCR on the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA). The 20 µL mixture reaction compressed 10 µL of 2× SYBR Green Master Mix, 1 µL of forward and reverse primers (10 µM each), 2 µL of diluted cDNA template, 6 µL of nuclease-free water. Thermal cycling comprised initial denaturation at 95°C for 3 min, followed by 40 cycles each of denaturation at 95°C for 15 sec, annealing/extension at 60°C for 30 sec, Each sample was run in triplicate, and non-template controls (NTC) were included to check for contamination. GAPDH was used as the housekeeping gene for mRNA and lncRNA normalization. U6 small nuclear RNA was used for miRNA normalization.

The relative gene expression was calculated using the 2^−ΔΔCt method (Livak and Schmittgen, 2001). Fold changes were expressed relative to the control group.

In silico *target prediction and validation*

Predicted miRNA–mRNA and lncRNA–mRNA interactions were retrieved from publicly available databases: miRWalk 3.0, TargetScan Human v8.0, miRTarBase, and lncBase v2. Only interactions with experimental validation and strong conservation across species were selected for biological validation.

Statistical analysis

All data were analyzed using IBM SPSS Statistics (Version 23, IBM Corp.) and GraphPad Prism (Version 9.0) for visualization. Results are presented as mean ± standard deviation (SD). Inter-group comparisons were made using one-way ANOVA, followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. Outliers and non-normal distributions were verified using Shapiro-Wilk test and Levene's test for homogeneity of variance.

RESULTS

Demographic and biochemical profile of patients

The study included a total of 150 individuals (aged 50–70 years), divided into three groups: DFU (n= 50), T2DM without complications (n= 50), and healthy controls (n = 50). Demographic and biochemical features, including lipid profile and HbA1c levels, were assessed and are summarized in Table I. Significant differences in HbA1c and triglyceride levels were observed among the groups, with DFU patients showing the highest HbA1c values, confirming poor glycemic control.

miRNA expression profiling and target gene analysis

Expression of three key miRNAs (hsa-miR-143-3p, hsa-miR-145-5p, and hsa-miR-146a) was measured using qRT-PCR in all participants. Expression levels were normalized using U6 RNA, and fold changes were calculated using the 2^−ΔΔCt method. All three miRNAs exhibited significant downregulation in DFU tissue samples compared to both T2DM and healthy control groups. The most substantial decrease was observed when comparing DFU with healthy individuals:

Table I. Clinical and biochemical characteristics of Healthy, T2DM and DFU subjects, and their association with lncRNA GAS5, lncRNA WAKMAR2, miRNA, and target gene expression levels.

Parameter	Healthy	T2DM	DFU	DFU vs healthy (p-value)	lncRNA GAS5 (p-value)	lncRNA WAK-MAR2 (p-value)	miRNA expression (Fold change vs Healthy/ DM) (p-value)	Target gene expression (Fold change vs Healthy/ DM) (p-value)
Age (Years)	51.92 ± 9.6	52.13 ± 6.8	58.67 ± 5.96	0.11	0.10	0.13	0.12	0.10
BMI (kg/m²)	23.99 ± 0.68	24.27 ± 0.71	23.99 ± 0.68	0.07	0.04	0.07	0.04	0.09
Systolic BP (mm Hg)	120.54 ± 15.8	125.3 ± 5.2	139.2 ± 5.04	0.06	0.05	0.04	0.05	0.08
Diastolic BP (mm Hg)	79.91 ± 12.5	84.97 ± 2.7	86.76 ± 8.59	0.09	0.08	0.09	0.08	0.09
HbA1c (%)	5.8 ± 2.3	7.7 ± 4.5	8.98 ± 3.79	0.02	0.00	0.04	0.00	0.04
BSF (mg/dL)	90.12 ± 4.33	146.34 ± 9.12	127 ± 6.41	0.04	0.05	0.06	0.05	0.06
BSR (mg/dL)	156 ± 5.46	210.22 ±4.55	287 ± 11.16	0.00	0.07	0.02	0.07	0.01
Cholesterol (mg/dL)	182.06 ± 38.9	212.56 ± 18.92	205.6 ± 5.19	0.00	0.03	0.04	00.8	0.04
LDL-C (mg/dL)	120.29 ± 41.3	129.82 ± 21.35	134.6 ± 4.73	0.00	0.16	0.08	0.08	0.08
HDL-C (mg/dL)	32.72 ± 10.0	45.12 ± 5.7	35.45 ± 4.76	0.05	0.05	0.04	0.05	0.04
TG (mg/dL)	118.09 ± 35.1	182.14 ± 15.41	155.1 ± 2.53	0.05	0.01	0.06	0.07	0.06
VLDL (mg/dL)	15.56 ± 2.34	23.67 ± 7.11	28.44 ± 8.43	0.09	0.11	0.07	0.10	0.07
Body Fat (%)	26.72 ± 11.3	34.23 ± 4.6	32.67 ± 5.8	0.01	007	0.06	0.01	0.02
Visceral Fat	8.85 ± 4.3	12.88 ± 7.4	10.85 ± 8.3	0.06	0.08	0.07	0.07	0.06
Smoking (Yes/No)	39/11	37/13	31/19	0.14	0.11	0.13	0.17	0.17
STAT1 correlation	—	—	—	< 0.05	0.61/0.02	—	—	—
NFκB correlation	—	—	—	< 0.05	—	-0.56/0.04	—	—
hsa-miR-143-3p	—	↓ 0.53-fold	↓ 5.41-fold	< 0.05	—	—	↓ 5.41/ 0.53	MAP3K7↑5.4/3.8
hsa-miR-145-5p	—	↓ 0.3-fold	↓ 1.6-fold	< 0.05	—	—	↓ 1.6 / 0.3	LOX ↑ 5.3/1.9
hsa-miR-146a	—	↓ 0.61-fold	↓ 2.68-fold	< 0.05	—	—	↓ 2.68 / 0.61	THBD↑1.1/0.2

hsa-miR-143-3p showed 0.53-fold decrease vs. T2DM, 5.41-fold decrease vs. healthy controls; hsa-miR-145-5p showed 0.30-fold decrease vs. T2DM, 1.60-fold decrease vs. healthy controls; which hsa-miR-146a showed 0.61-fold decrease vs. T2DM and 2.68-fold decrease vs. healthy controls. Target genes *MAP3K7* (miR-143-3p), *LOX* (miR-145-5p), and *THBD* (miR-146a) corresponding to the selected miRNAs were analyzed. All three targets showed upregulated expression in DFU tissues compared to controls: *MAP3K7* showed 3.8-fold increase vs. T2DM, 5.4-fold increase vs. healthy controls; *LOX* 1.9-fold increase vs. T2DM and 5.3-fold increase vs. healthy controls; *THBD* showed 0.2-fold increase vs. T2DM and 1.1-fold increase vs. healthy controls.

The expression difference between DFU and healthy controls was statistically significant for all three miRNAs and their corresponding target genes ($p < 0.05$, Table I). Regression analysis confirmed strong inverse correlations between each miRNA and its target gene, with a consistent pattern across all groups.

lncRNAs expression and target genes analysis

To complement miRNA profiling, we evaluated the expression of two lncRNAs (GAS5 and WAKMAR2) and their respective target genes (*STAT1* and *NF-κB*) samples. GAS5 was significantly upregulated in DFU patients compared to healthy controls: it showed 0.36-fold increase vs. T2DM (36% increase), and 0.19-fold increase vs. healthy controls ($p < 0.005$). WAKMAR2 was significantly downregulated in DFU patients showing 0.40-fold decrease (40% decrease) vs. T2DM and 0.49-fold decrease vs. healthy controls, ($p < 0.001$, statistical validation: $t = -3.1$; 95% CI: −3.02 to −0.5). NF-κB, a downstream inflammatory mediator and WAKMAR2 target, showed significant upregulation in DFU individuals *viz.* 2.3-fold increase vs. T2DM and 2.41-fold increase vs. healthy controls, ($p < 0.001$), STAT1, a GAS5-regulated transcription factor, exhibited reduced expression in DFU showing 0.76-fold decrease vs. T2DM and 0.81-fold

decrease vs. healthy controls, (p = 0.0001, t = 3.12; 95% CI: 0.02–0.88).

These findings support the inverse regulatory effect of lncRNAs on their targets. Correlation coefficients between WAKMAR2 and NF-κB, and GAS5 and STAT1, were consistent with known interactions reported in regulatory network databases (Table I). Expression changes were visualized in Figure 1.

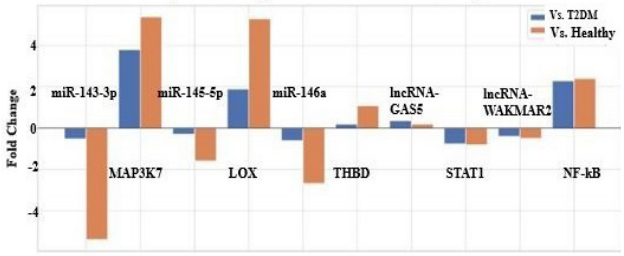

Fig. 1. Expression changes in DFU vs. T2DM and healthy controls.

When comparing DFU patients to T2DM patients without DFU, the following trends were noted: miRNAs showed moderate downregulation (0.3 to 0.6-fold decrease) in DFU. LncRNAs showed opposite regulation patterns, with GAS5 upregulated and WAKMAR2 downregulated. Target genes in both categories (MAP3K7, LOX, NF-κB) were significantly upregulated in DFU samples, confirming enhanced pro-inflammatory signaling and impaired wound healing mechanisms. THBD and STAT1, genes associated with anti-inflammatory or reparative pathways, were downregulated, consistent with poor re-epithelialization and chronic inflammation in DFU.

Statistical significance was established at $p < 0.05$ for all key comparisons. Expression values with $p < 0.001$ were considered highly significant. All qPCR data were normalized and calculated using the 2^−ΔΔCt method. T-tests and ANOVA confirmed significant differences across all comparisons; 95% confidence intervals (CI) were used to validate precision. Results are summarized in Table I, with graphical representations in Figure 1.

DISCUSSION

DFU represents a critical and complex complication of diabetes mellitus, involving a multifactorial pathology driven by metabolic dysregulation, impaired wound healing, and chronic inflammation. The current study aimed to evaluate the clinical, biochemical, and molecular alterations in DFU patients compared to diabetic individuals without complications (T2DM) and healthy controls. The combined analysis of clinical metrics, lipid profiles, miRNA expression, and their target gene regulation provided a holistic insight into disease pathophysiology. DFU arises from a multifaceted network of impaired inflammation, reduced angiogenesis, and dysfunctional tissue remodeling. Non-coding RNAs, including miRNAs and lncRNAs, are central in orchestrating these molecular events. This study extends our understanding by integrating both miRNA and lncRNA regulatory axes to provide a comprehensive molecular view of DFU. MiRNAs are fine-tuners of gene expression and their dysregulation is a hallmark of DFU. We observed a significant downregulation of miR-143, miR-145, and miR-146a in DFU tissue, which inversely correlated with increased expression of MAP3K7 (TAK1), LOX, and THBD all of which promote inflammatory and fibrotic signaling.

Statistical analysis revealed several parameters significantly associated with DFU compared to healthy individuals: DFU patients demonstrated significantly higher fasting (BSF= 127 mg/dL, p= 0.04) and random blood sugar levels (BSR= 287 mg/dL, $p < 0.001$), as well as elevated HbA1c (8.98%, p= 0.01). These indicators reflect poor glycemic control, which directly contributes to delayed wound healing and immune dysfunction in DFU. Both systolic (139.2 mm Hg, p= 0.02) and diastolic (86.76 mm Hg, p= 0.04) blood pressures were significantly elevated in DFU compared to healthy controls, suggesting hypertension as a compounding risk factor in vascular compromise and microcirculation damage. Total cholesterol (205.6 mg/dL), LDL-C (134.6 mg/dL), and triglycerides (155.1 mg/dL) were significantly increased in DFU patients ($p < 0.05$ for each), reflecting dyslipidemia. This lipid abnormality can impair endothelial function and promote inflammatory signaling key contributors to DFU progression. Although not reaching statistical significance (p= 0.06–0.08), increased visceral fat and body fat percentage in DFU patients indicate a metabolic syndrome-like profile, often associated with chronic inflammation and insulin resistance.

A central highlight of this study is the downregulation of three crucial miRNAs: hsa-miR-143-3p, hsa-miR-145-5p, and hsa-miR-146a in DFU tissue. These miRNAs are known regulators of inflammation, angiogenesis, and cellular stress responses. miR-143-3p showed a 5.41-fold decrease in DFU vs healthy ($p < 0.0001$). Its target gene MAP3K7 was upregulated by 5.4-fold, a kinase involved in inflammatory signaling through the NF-κB pathway. This inverse relationship suggests suppressed anti-inflammatory signaling and heightened cytokine activity in DFU. miR-145-5p downregulated by 1.6 fold in DFU ($p < 0.0005$). The target gene LOX, involved in extracellular matrix remodeling and fibrosis, was upregulated by 5.3 fold. These changes may contribute to aberrant wound

remodeling and excessive fibrotic deposition. miR-146a: Decreased by 2.68-fold ($p < 0.0003$). Its target THBD (thrombomodulin) was not significantly upregulated, implying that compensatory anti-inflammatory mechanisms may be failing in DFU pathology. The coordinated downregulation of miR-143-3p, miR-145-5p, and miR-146a, alongside upregulation of inflammatory and fibrosis-associated target genes (MAP3K7, LOX), points to a sustained pro-inflammatory environment in DFU. This environment not only delays wound healing but may predispose the tissue to necrosis, infection, and eventual amputation. Moreover, the strong correlation of lncRNA GAS5 with HbA1c ($r = 0.57$, $p= 0.00$) and BMI ($r= 0.49$, $p = 0.04$) further supports the hypothesis that non-coding RNAs play a pivotal role in glucose dysregulation and obesity-linked inflammatory signaling.

miR-146a acts as a molecular brake on NF-κB signaling by targeting TRAF6, IRAK1, and IRAK2. Its reduced expression in DFU prolongs inflammation and is consistent with earlier findings showing its downregulation in diabetic skin and rodent DFU models. The corresponding increase in THBD may serve to compensate for endothelial dysfunction. miR-143/145 pivotal in vascular function and tissue repair. Their downregulation promotes LOX-mediated matrix stiffening and MAP3K7-driven NF-κB activation. This aligns with literature indicating that their repression impedes angiogenesis and extracellular matrix integrity.

New therapeutics are emerging that target these miRNAs. For example, negative pressure wound therapy (NPWT) has been shown to suppress miR-155 to enhance healing (Huang *et al*., 2025), and exosomal delivery of miR-143-3p is being explored for pro-angiogenic effects. Furthermore, lncRNA-hosted miRNA networks such as NEAT1/miR-146a-5p are being investigated for their ability to rescue angiogenesis in DFU. LncRNAs as epigenetic regulators of wound healing. Our data revealed elevated expression of lncRNA GAS5 and diminished expression of WAKMAR2 in DFU patients, trends that were inversely correlated with their target genes: GAS5 upregulation coincided with reduced STAT1 expression. This axis is recognized for modulating macrophage polarization and fibrotic responses. GAS5 also activates the HIF1A-VEGF pathway via interaction with TAF15, which may explain its paradoxical elevation in hypoxic chronic wounds.

WAKMAR2 was significantly downregulated in DFU, which corresponded with heightened NF-κB expression confirming its barrier role against hyperinflammation. Notably, silencing WAKMAR2 disrupts keratinocyte migration and enhances inflammatory signaling. These findings align with a broader understanding that lncRNAs are crucial epigenetic regulators in DFU, as supported by emerging reviews highlighting miRNAs, lncRNAs, and circRNAs as key modulators of healing processes such as inflammation, re-epithelialization, and remodeling. Epigenetic mechanisms, such as DNA methylation and histone modification, act in concert with ncRNAs to modulate wound biology. A recent review emphasized how hyperglycemia-induced epigenetic alterations compound wound-chronicity by dysregulating ncRNAs and key growth factors like VEGF and TGF-β.

From a therapeutic standpoint, treatments like NPWT have demonstrated ncRNA-modulatory effects, notably through decreased miR-155 levels resulting in enhanced fibroblast function and angiogenesis (Huang *et al*., 2025). Additionally, innovative approaches, including exosomal delivery of miR-143, bioactive dressings loaded with ncRNAs, and CRISPR-mediated ncRNA editing, are emerging from recent preclinical studies. This study is limited by its cross-sectional design and single-biopsy sample set. Longitudinal analysis and multi-centric validation in larger cohorts (including females) are needed. Moreover, functional studies of these ncRNA interactions in vitro and in vivo are required to validate causality. Combined miRNA and lncRNA therapeutic modulation, delivered via exosomes or topical formulations, epigenome editing in wound-healing cells, personalized wound monitoring using ncRNA-based liquid biopsies, Integration with emerging bioinformatics pipelines to stratify patients and optimize ncRNA-based therapeutics.

CONCLUSION

Our study highlights the coordinated dysregulation of both miRNAs and lncRNAs in DFU, reinforcing their collective contribution to chronic inflammation, microvascular impairment, and ECM dysfunction. By integrating upstream (lncRNA) and downstream (miRNA) epigenetic regulators, this work provides a comprehensive framework for identifying novel biomarkers and targeted therapies with the potential to significantly improve DFU outcomes and reduce amputation rates. Clinical metrics reinforce the role of hyperglycemia and dyslipidemia; miRNA and target gene analysis expose key molecular dysfunctions; and non-coding RNA correlations highlight promising biomarkers for future diagnostic or therapeutic exploration. Further validation with larger cohorts and functional studies on identified miRNAs and their targets may pave the way for targeted molecular interventions in DFU management.

DECLARATIONS

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IRB approval

The study was conducted under ethical standards approved by the relevant institutional committee.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki (2013) and was approved by the Institutional Ethics Review Board of the Department of Biosciences, COMSATS University Islamabad, as well as the Ethics Committees of tertiary care hospitals involved in sample acquisition. Prior to participation, all individuals provided written informed consent after receiving comprehensive information regarding the purpose, procedures, and potential risks associated with the study.

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Generative AI and AI-assisted technology statement

No Generative AI or AI-assisted technologies were used in the creation of this manuscript. All content, analysis, and writing were performed by the authors.

Statement of conflict of interest

The authors have declared no conflict of interest.

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