Journal of Pioneering Medical Sciences
Received: August 13, 2026 | Accepted: October 29, 2026 | Published: November 15, 2026
Volume 15, Issue S02, Pages 218-222

DOI https://doi.org/10.47310/jpms202615S0225



Relative TNF-α Gene Expression in Edentulous Patients with and Without Diabetes Mellitus

Karthikeyan Murthykumar[1], Sarah Cherian[2], Dhanraj Ganapathy[3*] and J.Vijayashree Priyadharsini[4]

[1-2]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, India
[3]Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, India
[4]Professor, Clinical Genetics Lab, Centre for Cellular and Molecular Research, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai,Tamil Nadu, India

Author Designation: [1]Reader,[2]Undergraduate Student and [3]Professor and Head

*Corresponding author: Dhanraj Ganapathy (e-mail: dhanraj@saveetha.com).

©2026 the Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0

Abstract Objectives: Diabetes mellitus is associated with impaired wound healing, altered bone metabolism, and increased inflammatory activity, and has been linked to a higher prevalence of tooth loss and complete edentulism. Residual ridge resorption following tooth loss may be influenced by systemic metabolic conditions, with greater ridge resorption reported in diabetic individuals. Tumor necrosis factor-alpha (TNF-α), a major pro-inflammatory cytokine involved in immune regulation and bone remodeling, may contribute to persistent inflammation and altered tissue repair in diabetic patients. However, the expression of TNF-α in healed edentulous ridge tissues in diabetic individuals remains of clinical interest. **Aim:** To evaluate and compare TNF-α gene expression in edentulous patients with diabetes mellitus and edentulous patients without diabetes, and to assess its potential association with the persistent inflammatory environment of healed edentulous ridge tissues. **Methods:** Edentulous patients with diabetes mellitus constituted the case group, while edentulous patients without diabetes constituted the control group. Tissue samples were subjected to RNA isolation using TRIzol™ reagent, followed by complementary DNA synthesis. TNF-α gene expression was quantitatively assessed using real-time polymerase chain reaction (RT-qPCR) with TB Green chemistry. GAPDH was used as the internal control, and relative gene expression was evaluated using the comparative ΔΔCt method. **Results:** The mean relative TNF-α expression values of 3.41 in diabetic edentulous samples and 1.06 in non-diabetic samples (reported $p = 0.0106$). However, the number of biologically independent samples analysed, reference-gene performance, Ct/ΔCt values and the discrepancy between reported enrollment of 100 and a figure label of $n = 10$ per group remain unresolved. The statistical comparison therefore requires confirmation using the original RT-qPCR records. **Conclusion:** The analysis reported higher relative TNF-α gene-expression values in diabetic than in non-diabetic edentulous tissue samples. This molecular comparison does not establish the presence or magnitude of residual ridge resorption, prosthetic treatment failure, or a causal diabetes-related inflammatory mechanism, such hypotheses require clinical and longitudinal testing.

Key Words: Edentulism, Diabetes Mellitus, TNF-A, TNF-A Gene Expression, Tumor Necrosis Factor-Alpha, Residual Ridge Resorption, Post-Healing Residual Ridge Resorption, Edentulous Patients, Alveolar Bone Resorption, Inflammation, Bone Remodeling, Diabetic Wound Healing, RT-Qpcr, Prosthetic Rehabilitation, Infectious Disease, Public Health, Health Policy

INTRODUCTION

Diabetes mellitus is a major global public health concern. It is a chronic metabolic disorder characterized by hyperglycemia resulting from inadequate insulin secretion, impaired insulin action, or both. Diabetes is a chronic disease that occurs when the pancreas is not able to produce enough insulin or in cases when the body cannot effectively use the insulin produced by it. Insulin is a hormone that regulates blood glucose. Hyperglycaemia, also called raised blood glucose, is a common effect of diabetes that leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Diabetes mellitus has been associated with an increased risk of tooth loss and complete edentulism compared with individuals without diabetes[1]. Studies show that there is a rapid growth in diabetes seen in developing countries more in adults younger than 60 years, rather than in elderly people. The global prevalence of diabetes has increased substantially over recent decades, with the greatest increases occurring in low- and middle-income countries [2].

Edentulism or complete loss of teeth, is a common condition that significantly affects oral function, aesthetics, and overall quality of life. Edentulism can substantially impair mastication, speech, nutrition, and oral health-related quality of life [3]. Edentulism occurs because of biologic disease processes, such as dental caries, periodontal diseases, trauma, and oral cancer [4]. It is accompanied by several comorbidities that can significantly influence an individual. Although the rate of edentulism is declining, the number of edentulous patients is rising because of the increase in population. The management of edentulous patients has been addressed since the early days of dentistry. It is frequently associated with systemic diseases such as diabetes mellitus, which is known to influence wound healing, bone metabolism, and immune response. Diabetes mellitus has also been associated with altered oral conditions in complete denture wearers, emphasizing the importance of considering systemic metabolic status during prosthodontic management [5]. Previous studies involving diabetic completely edentulous patients have also demonstrated measurable biological differences relevant to prosthodontic and implant rehabilitation [6]. Among the various molecular mechanisms involved, inflammation plays a central role in both tooth loss and its complications. Diabetes may also influence the condition of the residual alveolar ridge after tooth loss. A case-control study of completely edentulous patients reported greater residual ridge resorption in diabetic individuals compared with nondiabetic individuals, particularly in association with poorer glycemic control [7]. Since TNF-α is an important mediator of inflammatory bone remodeling, increased TNF-α expression in diabetic patients may contribute to an altered inflammatory environment within the residual ridge and may have implications for subsequent prosthetic or implant rehabilitation. The relevance of diabetes to inflammatory complications associated with implant-supported prosthetic rehabilitation has also been investigated using molecular biomarkers, highlighting the importance of systemic metabolic status in prosthodontic treatment [8].

Tumor necrosis factor-alpha (TNF-α) is a key pro-inflammatory cytokine that regulates immune responses and bone remodeling. In oral inflammatory disease, elevated TNF-α levels have been demonstrated in patients with chronic periodontitis, supporting its role as an important mediator of periodontal tissue inflammation [9]. Increased expression of TNF-α has been linked to chronic inflammation, impaired bone healing, and periodontal tissue destruction, which are commonly observed in edentulous patients, particularly those with poorly controlled diabetes. TNF-α regulates immune responses and bone remodeling. Increased expression of TNF-α has been linked to chronic inflammation, impaired bone healing, and periodontal tissue destruction, particularly in diabetes-associated periodontal disease [10]. TNF-α is primarily generated in adipocytes and/or peripheral tissues, and it triggers tissue-specific inflammation by facilitating ROS production and activating several transcriptional pathways. TNF-α has been implicated in insulin resistance by disrupting insulin signaling, including through serine phosphorylation of insulin receptor substrate-1 (IRS-1) [11] .It is functionally known to trigger a series of various inflammatory molecules, including other cytokines and chemokines. TNF-α exists in a soluble and transmembrane form. The transmembrane TNF-α (tmTNF-α) is the initially synthesized precursor form and is required to be processed by TNF-α-converting enzyme (TACE), a membrane-bound disintegrin metalloproteinase, to be released as the soluble TNF-α (sTNF-α).TNF-α exists in both transmembrane and soluble forms, the transmembrane precursor can be cleaved by TNF-α-converting enzyme (TACE/ADAM17) to generate soluble TNF-α [12].The pathogenesis of type 2 diabetes mellitus (T2DM) and the onset of insulin resistance involve multiple stimulatory factors, particularly glucolipotoxicity, production of reactive oxygen species (ROS), epigenetic influences, activation of several transcriptionally mediated pathways, and increased levels of various pro-inflammatory cytokines. Among the other pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) is one of the key pro-inflammatory mediators that plays a crucial role in the onset of insulin resistance and the development of T2DM.Recent molecular studies from oral tissues have further demonstrated differential TNF-α gene expression, supporting the relevance of TNF-α as a molecular marker of altered inflammatory activity in oral disease [13]. This study aims to evaluate and compare the gene expression levels of TNF-α in edentulous patients with and without diabetes, offering a better understanding of the molecular interactions that contribute to oral health complications in diabetic individuals. Therefore, this study aimed to evaluate and compare TNF-α gene expression in edentulous patients with and without diabetes mellitus and to explore its potential role in the inflammatory environment of healed edentulous ridge tissues.

METHODS

Study Population

A total of 100 edentulous patients were enrolled in this study, comprising both males and females. Participants were divided into two groups: diabetic edentulous patients and non-diabetic edentulous controls. Patients with diabetes were included based on confirmed diagnosis and HbA1c levels, whereas individuals with other systemic illnesses, smoking habits, or alcohol consumption were excluded to eliminate potential confounding factors. Written informed consent was obtained from all subjects prior to sample collection.

Sample Collection

Soft tissue specimens were obtained from the alveolar mucosa during routine prosthodontic procedures. Approximately 2–3 mm tissue samples were collected under aseptic conditions and immediately stored in RNAlater™ solution (Thermo Fisher Scientific, USA) at –80°C until further processing.

Table 1: PCR Reaction Mixture

Reagent	Volume	Concentration
qPCR Premix (2X)	10 µl	1X
qPCR Forward Primer (10 µM)	0.4 µl	0.2 µM
qPCR Reverse Primer (10 µM)	0.4 µl	0.2 µM
Template	2 µl	100 ng
dH_2O	6.8 µl	—
Total	20 µl	—

Table 2: Reaction Setup

Reagent	Volume	Concentration
qPCR Premix (2X)	10 µl	1X
qPCR Forward Primer (10 µM)	0.4 µl	0.2 µM
qPCR Reverse Primer (10 µM)	0.4 µl	0.2 µM
Template	2 µl	100 ng
dH_2O	6.8 µl	—
Total	20 µl	—

Table 3: Reaction Setup

Primer	Forward (5′–3′)	Reverse (5′–3′)
TNF-α	TCCACCG...GACTCCTAC	CCTCCC...GATGGCTCA

RNA Isolation

To isolate RNA, add 1 mL of TRIzol™ Reagent to the tissue and homogenize using a homogenizer. Add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent used, securely cap the tube, and mix thoroughly by shaking. Incubate the sample for 2–3 minutes, then centrifuge for 15 minutes at 12,000 × g at 4°C. This step separates the mixture into a lower phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. Carefully transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out. Add 0.5 mL of isopropanol per 1 mL of TRIzol™ Reagent used for lysis, then incubate the mixture for 10 minutes at 4°C. Centrifuge the sample for 10 minutes at 12,000 × g at 4°C. A white gel-like RNA pellet will form at the bottom of the tube. Discard the supernatant with a micropipette. To precipitate the RNA, resuspend the pellet in 1 mL of 75% ethanol. Vortex the sample briefly and centrifuge for 5 minutes at 7500 × g at 4°C. Discard the supernatant with a micropipette, then vacuum or air-dry the RNA pellet for 5–10 minutes. Resuspend the pellet in 20–50 µL of Nuclease-free water, by pipetting up and down. The RNA can be stored at –70°C for later use.

cDNA Conversion

To synthesize cDNA using the iScript™ cDNA Synthesis Kit, prepare a 20 µl reaction by combining 4 µl of 5x iScript Reaction Mix, 1 µl of iScript Reverse Transcriptase, 500ng of RNA template, and nuclease-free water to adjust the total volume. Incubate the reaction mix in a thermal cycler under the following conditions: priming at 25°C for 5 minutes, reverse transcription at 46°C for 20 minutes, and reverse transcriptase inactivation at 95°C for 1-minute Table 1-2.

RT PCR

Real-time PCR was performed using TBgreen qPCR premixes (Takara Biotechnology, China) on CFX Opus Real-Time PCR Systems (Biorad). The primer, components, and reaction conditions were tabulated. Relative TNF-α gene expression was calculated using the comparative 2^–ΔΔCt method, with GAPDH used as the endogenous internal control Table 3.

RESULTS

The reported group means of 3.41 and 1.06 should be interpreted as relative expression rather than absolute mRNA concentration. For reproducibility, results need the number of biological specimens analysed per group, reference-gene stability assessment, Ct/ΔCt/ΔΔCt distributions, amplification efficiencies and documented reaction replicates. Figure sample-size labels must match the actual analysed specimens, the displayed n = 10 label is inconsistent with the reported enrollment of 100 participants.

Table 4: TNF Alpha Results

Group	Mean Expression (2^-ΔΔCT)
CASE	3.41
CONTROL	1.06
p-value (t-test)	0.0106

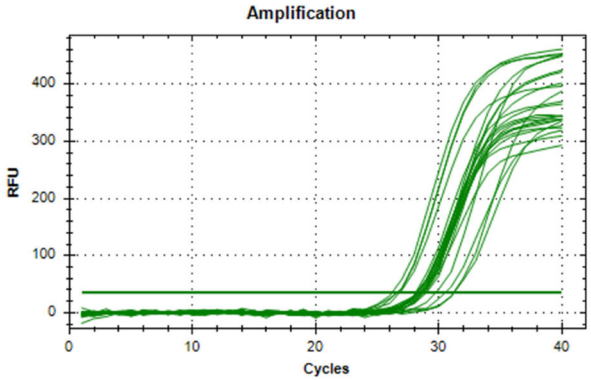


Figure 1: Amplification Plot Showing TNF-A Gene Expression in Diabetic and Non-Diabetic Edentulous Patients

TNF-α gene expression was significantly higher in edentulous patients with diabetes mellitus compared with edentulous patients without diabetes (Table 4). The mean relative TNF-α gene expression was 3.41 in the diabetic group and 1.06 in the non-diabetic group. The difference between the groups was statistically significant (p = 0.0106).

The amplification plot demonstrates the quantitative measurement of TNF-α gene expression during the real-time PCR process. In this plot, the fluorescence signal representing the amplified gene product is plotted against the PCR cycles. The case group, comprising edentulous patients with diabetes, shows earlier amplification, as evidenced by the curve crossing the threshold at lower cycle numbers. This indicates that the TNF-α gene was present in higher quantities at the start of the reaction, reflecting increased gene expression in the diabetic samples. In contrast, the control group, consisting of edentulous patients without diabetes, shows amplification occurring at a later stage with a gentler slope, suggesting lower levels of the gene transcript. The amplification difference observed between the two groups implies that chronic hyperglycemia and systemic inflammation in diabetic patients may upregulate TNF-α gene expression, even in healed edentulous sites Figure 1.

Expression Box Plot of TNF-α

The box plot demonstrated higher TNF-α gene expression in the diabetic edentulous group compared with the non-diabetic group. The diabetic group showed a higher median expression and greater variability in TNF-α expression, whereas the non-diabetic group showed lower expression

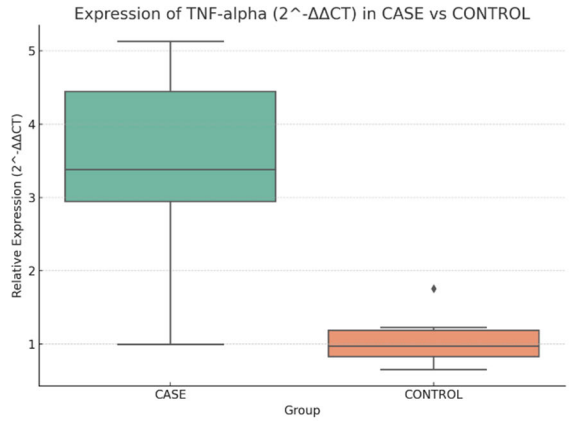


Figure 2: Box Plot Showing the Distribution of Relative TNF-A Gene Expression in Diabetic and Non-Diabetic Edentulous Patients

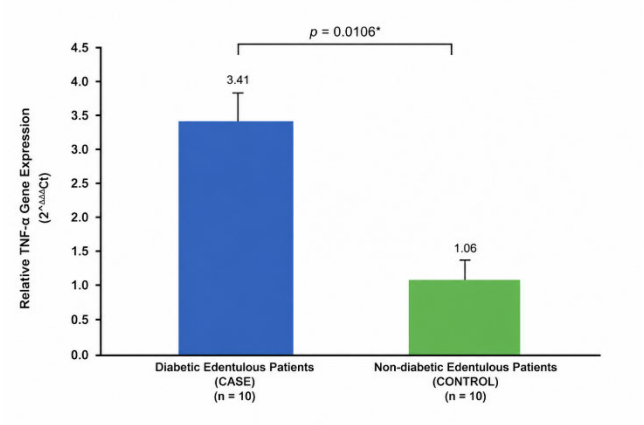


Figure 3: Bar Graph Showing Mean Relative TNF-A Gene Expression in Diabetic and Non-Diabetic Edentulous Patients

with a narrower distribution. The observed difference between the groups was statistically significant ($p<0.05$) Figure 2.

TNF-α Expression

The bar graph demonstrated higher mean TNF-α gene expression in the diabetic group (3.41) compared with the non-diabetic group (1.06). The difference between the groups was statistically significant ($p = 0.0106$) Figure 3.

DISCUSSION

The present study demonstrated significantly higher TNF-α gene expression in edentulous patients with diabetes mellitus compared with non-diabetic edentulous patients. This increased expression may indicate a persistent pro-inflammatory environment in the healed edentulous ridge tissues of patients with diabetes mellitus. These findings are consistent with previous experimental evidence demonstrating increased inflammatory cytokine expression in diabetes. In a study using diabetic rats, periodontal tissues showed increased expression of pro-inflammatory cytokines, with significantly increased TNF-α-positive cells following diabetes induction [14]. Similarly, wounds in diabetic mice demonstrated approximately threefold higher TNF-α mRNA and protein levels than normoglycemic controls, indicating persistent TNF-α-associated inflammation during the healing process [15] .Previous periodontal studies have also reported elevated local pro-inflammatory cytokines, including IL-1β and IL-6, in periodontal disease [16] .Elevated TNF-α may contribute to delayed tissue healing and altered bone remodeling in diabetes. Increased inflammatory activity has also been associated with peri-implant tissue inflammation [17].The elevated TNF-α expression observed in the diabetic group may be related to the impaired healing response associated with diabetes. Diabetes is characterized by prolonged inflammation and altered cellular responses during wound repair, and increased TNF-α expression has been associated with delayed healing [18]. In the oral cavity, diabetes can affect both soft- and hard-tissue healing by altering inflammatory responses and reducing connective tissue and bone formation [19]. Therefore, the increased TNF-α expression observed in the present study may reflect persistent inflammatory activity in the healed edentulous ridge.

Increased inflammatory activity may influence tissue repair and bone remodeling in diabetic edentulous patients. The variability in TNF-α expression observed among diabetic patients may reflect differences in metabolic control or other patient-related factors, however, these factors were not directly evaluated in the present study.

The approximately 3.2-fold higher mean TNF-α gene expression observed in the diabetic group represents a significant difference between the two groups. TNF-α is an important pro-inflammatory cytokine involved in immune regulation, tissue inflammation, and bone remodeling. The increased expression observed in diabetic edentulous patients may therefore have implications for post-healing tissue remodeling and residual ridge changes.

Overall, the present findings suggest that diabetes mellitus is associated with increased TNF-α gene expression in healed edentulous ridge tissues. This increased expression may have implications for tissue remodeling and post-healing residual ridge changes. These findings are consistent with previous evidence showing altered expression of inflammatory mediators, including TNF-α, IL-6, and IL-8, in periodontal and peri-implant tissues associated with diabetes [20-22]. Further studies assessing glycemic control, duration of diabetes, and quantitative residual ridge resorption alongside TNF-α expression are needed to clarify this relationship.

CONCLUSIONS

This exploratory tissue-expression comparison cannot independently demonstrate altered bone remodeling or residual ridge resorption. Interpretation should remain limited to the measured relative transcript signal until tissue sourcing, assay specificity and specimen-level data are confirmed.

Diabetes mellitus is associated with significantly increased TNF-α gene expression in edentulous ridge tissues, suggesting persistence of an inflammatory response even after healing. Elevated TNF-α expression may contribute to altered bone remodeling, post-healing residual ridge resorption, impaired tissue repair, and challenges associated with prosthetic rehabilitation in diabetic edentulous patients. TNF-α may therefore have potential as a molecular marker for understanding the biological changes occurring in the healed edentulous ridge in patients with diabetes.

REFERENCES

[1] Žiūkaitė, L. *et al.* "Edentulism among diabetic patients compared to non-diabetic controls: a systematic review and meta-analysis." International Journal of Dental Hygiene, vol. 22, no. 1, 2024, pp. 3–14. https://doi.org/10.1111/idh.12762

[2] NCD Risk Factor Collaboration (NCD-RisC). "Worldwide trends in diabetes prevalence and treatment from 1990 to 2022: a pooled analysis of 1108 population-representative studies with 141 million participants." The Lancet, vol. 404, no. 10467, 2024, pp. 2077–2093. https://doi.org/10.1016/S0140-6736(24)02317-1

[3] Polzer, I. *et al.* "Edentulism as part of the general health problems of elderly adults." International Dental Journal, vol. 60, no. 3, 2010, pp. 143–155.

[4] Al-Rafee, M.A. "The epidemiology of edentulism and the associated factors: a literature review." Journal of Family Medicine and Primary Care, vol. 9, no. 4, 2020, pp. 1841–1843. https://doi.org/10.4103/jfmpc.jfmpc_1181_19

[5] Ganapathy, D.M. *et al.* "Evaluation of the influence of blood glucose level on oral candidal colonization in complete denture wearers with type-II diabetes mellitus: an in vivo study." Dental Research Journal (Isfahan), vol. 10, no. 1, 2013, pp. 87–92. https://doi.org/10.4103/1735-3327.111806

[6] George, R. *et al.* "Estimation of L-carnitine levels in diabetic completely edentulous patients for implant diagnosis: a cross-sectional study." Dental Research Journal (Isfahan), vol. 20, 2023, article 96.

[7] Mistry, R.A. *et al.* "An appraisal of bone resorption in completely edentulous diabetic and nondiabetic patients at prospective implant site in anterior mandible using digital volumetric tomography and its correlation with glycemic control: a case-control study." National Journal of Maxillofacial Surgery, vol. 11, no. 1, 2020, pp. 28–33. https://doi.org/10.4103/njms.NJMS_100_19

[8] Fathima, F. *et al.* "Evaluation of salivary caspase-3 as a biomarker for peri-implantitis in type 2 diabetic patients with full-arch implant-supported prostheses: a cross-sectional study." The Journal of Contemporary Dental Practice, vol. 26, no. 6, 2025, pp. 551–554. https://doi.org/10.5005/jp-journals-10024-3849

[9] Varghese, S.S. *et al.* "Estimation of salivary tumor necrosis factor-alpha in chronic and aggressive periodontitis patients." Contemporary Clinical Dentistry, vol. 6, suppl. 1, 2015, pp. S152–S156. https://doi.org/10.4103/0976-237X.166816

[10] Graves, D.T. *et al.* "Evidence that diabetes mellitus aggravates periodontal diseases and modifies the response to an oral pathogen in animal models." Compendium of Continuing Education in Dentistry, vol. 25, no. 7, suppl. 1, 2004, pp. 38–45.

[11] Hotamisligil, G.S. *et al.* "IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance." Science, vol. 271, no. 5249, 1996, pp. 665–668. https://doi.org/10.1126/science.271.5249.665

[12] Qu, Y. *et al.* "Forward and reverse signaling mediated by transmembrane tumor necrosis factor-alpha and TNF receptor 2: potential roles in an immunosuppressive tumor microenvironment." Frontiers in Immunology, vol. 8, 2017, article 1675. https://doi.org/10.3389/fimmu.2017.01675

[13] Varshan, M.S. *et al.* "MicroRNA-34a-3p and its target tumor necrosis factor-α in the regulation of South Indian oral squamous cell carcinoma population." Minerva Dental and Oral Science, vol. 73, no. 5, 2024, pp. 256–263. https://doi.org/10.23736/S2724-6329.23.04835-0

[14] Claudino, M. *et al.* "Spontaneous periodontitis development in diabetic rats involves an unrestricted expression of inflammatory cytokines and tissue destructive factors in the absence of major changes in commensal oral microbiota." Experimental Diabetes Research, vol. 2012, 2012, article 356841. https://doi.org/10.1155/2012/356841

[15] Siqueira, M.F. *et al.* "Impaired wound healing in mouse models of diabetes is mediated by TNF-alpha dysregulation and associated with enhanced activation of forkhead box O1 (FOXO1)." Diabetologia, vol. 53, no. 2, 2010, pp. 378–388. https://doi.org/10.1007/s00125-009-1529-y

[16] Becerik, S. *et al.* "Gingival crevicular fluid and plasma acute-phase cytokine levels in different periodontal diseases." Journal of Periodontology, vol. 83, no. 10, 2012, pp. 1304–1313. https://doi.org/10.1902/jop.2012.110616

[17] Liu, R. *et al.* "Tumor necrosis factor-alpha mediates diabetes-enhanced apoptosis of matrix-producing cells and impairs diabetic healing." The American Journal of Pathology, vol. 168, no. 3, 2006, pp. 757–764. https://doi.org/10.2353/ajpath.2006.050907

[18] Xu, F. *et al.* "Abnormal cell responses and role of TNF-α in impaired diabetic wound healing." BioMed Research International, vol. 2013, 2013, article 754802. https://doi.org/10.1155/2013/754802

[19] Ko, K.I. *et al.* "Diabetic wound healing in soft and hard oral tissues." Translational Research, vol. 236, 2021, pp. 72–86. https://doi.org/10.1016/j.trsl.2021.05.001

[20] Venza, I. *et al.* "Proinflammatory gene expression at chronic periodontitis and peri-implantitis sites in patients with or without type 2 diabetes." Journal of Periodontology, vol. 81, no. 1, 2010, pp. 99–108. https://doi.org/10.1902/jop.2009.090358

[21] Roy, A. and Preetha, S. "Exploration of potent compounds with glucokinase activity for diabetes through molecular docking analysis." Research Journal of Pharmacy and Technology, vol. 17, no. 12, 2024, pp. 5981–5983.

[22] Subramaniam, S. *et al.* "An investigation on antidiabetic competence of Ferula assafoetida mediated AgNPs on diabetic (STZ) induced albino rats." Biocatalysis and Agricultural Biotechnology, vol. 56, 2024, article 103043.