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Evaluation and Comparison of Salivary Annexin-1 Levels in Type-2 Diabetic Patients with and without Generalized Periodontitis

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Abstract: This study evaluates and compares salivary Annexin-1 levels in Type-2 diabetic patients with and without generalized periodontitis. Saliva samples from 100 participants were analyzed using ELISA. The results indicated significantly lower Annexin-1 levels in diabetic patients with periodontitis compared to those without. These findings suggest that decreased Annexin-1 levels are associated with periodontal inflammation in Type-2 diabetic patients, highlighting its potential as a biomarker for periodontal disease in this population. The objective of this study is to evaluate and compare salivary Annexin-1 levels in Type-2 diabetic patients with and without generalized periodontitis to elucidate the relationship between this inflammatory biomarker and periodontal disease in the context of diabetes. In the methods section, a cross-sectional study was conducted involving two groups of Type-2 diabetic patients: those with generalized periodontitis (Group A) and those without periodontitis (Group B). Unstimulated saliva samples were collected from all participants, and Annexin-1 levels were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed to compare Annexin-1 levels between the groups and to explore correlations with clinical periodontal parameters and glycaemic control. The results included 50 participants in each group. Salivary Annexin-1 levels were significantly lower in Group A compared to Group B ($p<0.05$). Annexin-1 levels were negatively correlated with probing depth and clinical attachment loss but showed no significant correlation with HbA1c levels. In conclusion, the findings suggest that decreased salivary Annexin-1 levels are associated with generalized periodontitis in Type-2 diabetic patients. Annexin-1 may serve as a potential biomarker for inflammation and periodontal disease in this population.

Keywords: Type-2 Diabetes Mellitus, Periodontitis, Annexin-1, Salivary Biomarkers, Inflammation, ELISA, Diabetic Complications

1. Introduction

Type-2 diabetes mellitus (T2DM) is a prevalent chronic metabolic disorder that has reached epidemic proportions globally, affecting millions of people. It is characterized by insulin resistance, relative insulin deficiency, and hyperglycemia. The chronic hyperglycaemic state of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. T2DM is also associated with an increased susceptibility to infections and inflammatory conditions, including periodontal disease. Periodontal disease, particularly generalized periodontitis, is a chronic inflammatory condition that affects the supporting structures of the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone. It is one of the most common oral diseases, leading to tooth loss if left

untreated [2]. The relationship between diabetes and periodontal disease has been extensively studied. Evidence suggests a bidirectional link between the two conditions, where diabetes increases the risk and severity of periodontal disease, and periodontal disease negatively impacts glycemic control [3]. The hyperglycaemic environment in diabetic patients contributes to the accumulation of advanced glycation end products (AGEs), which promote the release of pro-inflammatory cytokines and reactive oxygen species (ROS) [4]. This results in an exaggerated inflammatory response, which can enhance tissue destruction and impair repair processes in the periodontium. Annexin-1 is a protein that has emerged as a significant player in the resolution of inflammation [5]. It belongs to the annexin family of calcium and phospholipid-binding proteins and is known for its anti-inflammatory properties. Annexin-1 inhibits leukocyte migration, promotes the clearance of apoptotic cells by macrophages, and modulates the production of pro-inflammatory mediators [6]. Given its role in controlling inflammation, Annexin-1 has been studied in various inflammatory and autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease. The potential role of Annexin-1 in periodontal disease and its relationship with diabetes has garnered research interest [7]. Periodontal disease is characterized by chronic inflammation and the presence of pathogenic microorganisms that stimulate an immune response. The resolution of inflammation is crucial in preventing the progression of periodontal disease, and proteins like Annexin-1 may play a key role in this process [8]. In diabetic patients, the impaired resolution of inflammation could contribute to the increased prevalence and severity of periodontal disease observed in this population [9]. Saliva, a readily accessible and non-invasive diagnostic fluid, contains a variety of biomarkers that reflect the physiological and pathological state of the body [10].

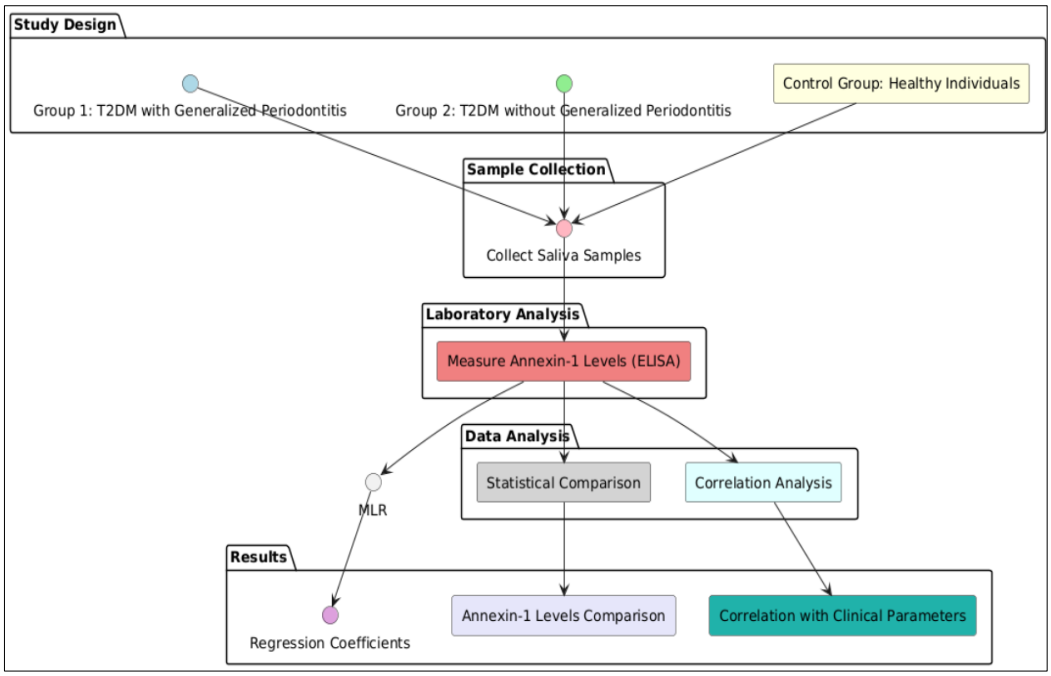

Figure 1. Basic Block Diagram of Evaluation of Type-2 diabetes mellitus (T2DM)

The use of salivary biomarkers for the diagnosis and monitoring of systemic and oral diseases has gained attention due to the ease of collection and the potential to provide real-time information about disease status. Salivary Annexin-1 levels could potentially serve as a biomarker for periodontal inflammation and disease activity, particularly in the context of diabetes [11]. This study aims to evaluate and compare the salivary levels of Annexin-1 in Type-2 diabetic patients with and without generalized periodontitis [12]. Understanding the interplay between Annexin-1, diabetes, and periodontal disease may provide insights into the inflammatory mechanisms underlying these conditions and inform clinical management strategies. Specifically, the study seeks to determine whether salivary Annexin-1 levels differ significantly between diabetic patients with generalized periodontitis and those without periodontitis, and to explore the potential correlations between Annexin-1 levels (As shown in Figure 1), periodontal parameters, and glycemic control [13]. The hypothesis of this study is that salivary Annexin-1 levels will be lower in Type-2 diabetic patients with generalized periodontitis compared to those without periodontitis. This hypothesis is based on the premise that the chronic inflammatory state associated with periodontitis may lead to decreased levels of Annexin-1, reflecting an impaired resolution of inflammation [14]. It is hypothesized that there will be a negative correlation between Annexin-1 levels and clinical parameters of periodontal disease, such as probing depth and clinical attachment loss, indicating that lower levels of Annexin-1 are associated with more severe periodontal inflammation and tissue destruction [15]. The objectives of this

study are threefold, (1) to compare the salivary levels of Annexin-1 between Type-2 diabetic patients with generalized periodontitis and those without periodontitis; (2) to explore the correlations between salivary Annexin-1 levels and clinical periodontal parameters; and (3) to assess the relationship between salivary Annexin-1 levels and glycemic control, as measured by HbA1c levels [16]. To achieve these objectives, a cross-sectional study design will be employed, involving the recruitment of Type-2 diabetic patients with and without generalized periodontitis. Comprehensive periodontal examinations will be conducted to diagnose and classify the periodontal status of the participants [17]. Unstimulated saliva samples will be collected and analyzed for Annexin-1 levels using enzyme-linked immunosorbent assay (ELISA). Statistical analyses will be performed to compare Annexin-1 levels between the two groups and to explore correlations with periodontal and glycemic parameters. This study is significant because it addresses a gap in the current understanding of the role of Annexin-1 in the context of diabetes and periodontal disease. By elucidating the relationship between salivary Annexin-1 levels and periodontal inflammation in diabetic patients, the findings may contribute to the development of novel diagnostic and therapeutic approaches for managing periodontal disease in this population [18]. Identifying salivary biomarkers that reflect the inflammatory status of the periodontium could enhance the ability to monitor disease progression and response to treatment in a non-invasive manner [19]. The interplay between diabetes, periodontal disease, and inflammation is complex and multifaceted. Annexin-1, with its anti-inflammatory properties, represents a potential biomarker for assessing periodontal inflammation and disease activity in diabetic patients. This study aims to provide valuable insights into the levels of salivary Annexin-1 in Type-2 diabetic patients with and without generalized periodontitis, shedding light on its potential role in the pathogenesis and management of periodontal disease in the context of diabetes [20].

2. Materials and Methods

Participants were recruited from the outpatient department of a tertiary care hospital. Informed consent was obtained from all participants. The diagnosis of T2DM was based on the American Diabetes Association criteria, and generalized periodontitis was diagnosed according to the criteria of the American Academy of Periodontology.

A. Material

This study employs a cross-sectional design to evaluate and compare the salivary levels of Annexin-1 in Type-2 diabetic patients with and without generalized periodontitis. A cross-sectional design is appropriate for identifying associations between variables at a single point in time, making it suitable for comparing biomarker levels between groups and exploring potential correlations with clinical parameters. The study was conducted in compliance with ethical standards and received approval from the institutional review board. Informed consent was obtained from all participants prior to enrolment.

- **Inclusion Criteria**

Participants were selected based on the following criteria,

- Age: Participants were aged between 35 and 65 years to control for age-related variations in periodontal status and salivary biomarker levels.
- Diagnosis of Type-2 Diabetes Mellitus: All participants had a confirmed diagnosis of Type-2 diabetes mellitus based on the American Diabetes Association (ADA) criteria, which include a fasting plasma glucose level of ≥126 mg/dL, a 2-hour plasma glucose level of ≥200 mg/dL during an oral glucose tolerance test, or an HbA1c level of ≥6.5%.
- Duration of Diabetes: Participants had a duration of diabetes of at least 5 years to ensure a well-established condition that could potentially influence periodontal health.
- Periodontal Status: Group A (Generalized Periodontitis): Participants in this group had generalized periodontitis, defined by the presence of probing depth (PD) ≥4 mm and clinical attachment loss (CAL) ≥3 mm affecting more than 30% of sites. Group B (No Periodontitis): Participants in this group had no signs of periodontitis, with PD <4 mm and CAL <3 mm at all sites.

- **Exclusion Criteria**

Participants were selected based on the following criteria,

- Other Systemic Diseases: Participants with systemic conditions other than diabetes that could affect periodontal health or salivary biomarkers (e.g., autoimmune diseases, chronic inflammatory conditions) were excluded.
- Smoking: Current smokers and those who had quit smoking within the past year were excluded due to the well-known impact of smoking on periodontal health and inflammation.

- Medications: Participants taking medications known to affect periodontal health or inflammation (e.g., corticosteroids, immunosuppressants) were excluded to avoid confounding effects.
- Pregnancy: Pregnant women were excluded due to hormonal changes that can affect periodontal health and salivary composition.

Participants were recruited from a diabetes clinic affiliated with a tertiary care hospital. Flyers and posters were placed in the clinic to inform potential participants about the study. Clinicians at the clinic referred eligible patients to the study team. Interested individuals underwent an initial screening to confirm their eligibility based on the inclusion and exclusion criteria.

Criteria	Group A (Generalized Periodontitis)	Group B (No Periodontitis)	Justification
Age Range (years)	35-65	35-65	Age range chosen to control for age-related variations in periodontal status and salivary biomarker levels.
Diagnosis of T2DM	Confirmed diagnosis based on ADA criteria	Confirmed diagnosis based on ADA criteria	Ensures all participants meet standardized criteria for Type-2 diabetes mellitus diagnosis.
Duration of Diabetes	≥5 years	≥5 years	Longer duration of diabetes selected to capture well-established conditions that may influence periodontal health.
Periodontal Status	Probing Depth (PD) ≥4 mm and Clinical Attachment Loss (CAL) ≥3 mm affecting >30% of sites	PD <4 mm and CAL <3 mm at all sites	Criteria used to distinguish between groups with and without generalized periodontitis.
Exclusion Criteria	Excludes smokers, individuals with other systemic diseases, and those taking medications affecting periodontal health	Excludes smokers, individuals with other systemic diseases, and those taking medications affecting periodontal health	Ensures study groups are comparable and reduces confounding factors that could influence periodontal and salivary biomarker levels.

Table 1. Study Design and Participant Selection Criteria

In this Table 1, outlines the criteria used for selecting participants in the study comparing salivary Annexin-1 levels in Type-2 diabetic patients with and without generalized periodontitis. Criteria include age range, diagnosis of Type-2 diabetes mellitus (T2DM) based on ADA criteria, duration of diabetes, periodontal status defined by probing depth and clinical attachment loss, and exclusion criteria to minimize confounding factors.

B. Method

Unstimulated saliva samples were collected from all participants in the morning, at least 2 hours after eating or drinking, to avoid diurnal variation. Participants were instructed to rinse their mouths with water and then relax for 5 minutes before sample collection.

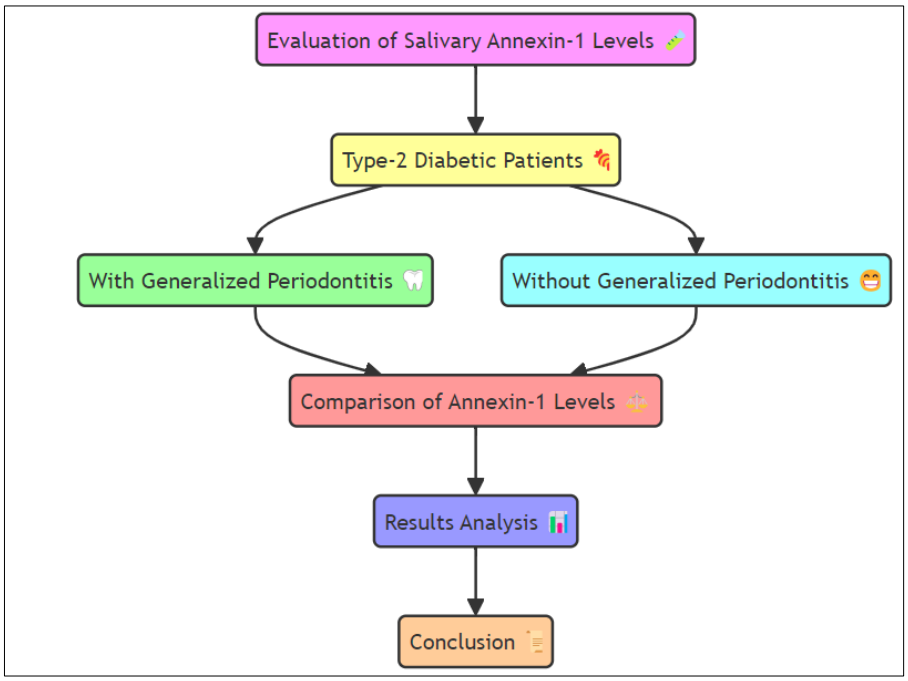

Figure 2. Basic Flow Analysis of Analysis of Type-2 Diabetes

Approximately 2-3 mL of saliva was collected in sterile tubes and immediately placed on ice. The samples were centrifuged at 3000 rpm for 10 minutes to remove debris and cellular components and then stored at -80°C until analysis.

Step 1]. Sample Collection and Analysis

To ensure the consistency and reliability of salivary Annexin-1 measurements, unstimulated saliva samples were collected from all participants between 8:00 AM and 10:00 AM. This time frame was chosen to minimize the effects of diurnal variations on salivary composition. Participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene procedures for at least one hour before sample collection to avoid contamination and ensure the purity of the saliva samples. Participants were asked to sit comfortably in a quiet room and to relax for a few minutes before sample collection. They were then instructed to allow saliva to accumulate in their mouths without stimulation (e.g., chewing, talking) and to gently spit the saliva into a sterile collection tube over a period of 5 minutes. The collection tubes were pre-labeled with unique identification codes to ensure proper tracking and analysis of the samples (As shown in Figure 2). Immediately after collection, the saliva samples were placed on ice to prevent degradation of proteins and other biomolecules. The samples were then transported to the laboratory for processing. Upon arrival, the samples were centrifuged at 3000 rpm for 15 minutes at 4°C to separate the supernatant from the cellular debris and mucus. This step was crucial for obtaining a clear and cell-free saliva sample suitable for subsequent biochemical analyses. The clear supernatant was carefully pipetted into sterile microcentrifuge tubes, ensuring that no cellular debris was carried over. The aliquoted saliva samples were then stored at -80°C until analysis. This low-temperature storage was necessary to preserve the integrity of Annexin-1 and other proteins, preventing enzymatic degradation and ensuring accurate measurement during the analysis phase.

Step 2]. Measurement of Salivary Annexin-1 Levels

Salivary Annexin-1 levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Annexin-1 ELISA Kit, ABC Biotech). The ELISA method was chosen for its sensitivity, specificity, and ability to quantitatively measure protein levels in biological samples. The assay was performed according to the manufacturer's instructions, with the following steps

- Preparation of Standards and Samples: Standards with known concentrations of Annexin-1 and saliva samples were brought to room temperature. The standards were used to generate a standard curve for quantification.
- Coating: ELISA plates pre-coated with an anti-Annexin-1 antibody were used. Wells designated for standards, samples, and blanks were filled accordingly.
- Incubation: The plates were incubated at room temperature for 1 hour to allow Annexin-1 in the samples and standards to bind to the immobilized antibodies.

- Washing: The plates were washed multiple times with a buffer solution to remove unbound substances.
- Detection Antibody: A biotinylated detection antibody specific for Annexin-1 was added to each well and incubated for another hour.
- Streptavidin-HRP: Following another wash step, streptavidin conjugated to horseradish peroxidase (HRP) was added to the wells and incubated for 30 minutes.
- Substrate Solution: A substrate solution was added to the wells, initiating a colorimetric reaction catalyzed by the HRP enzyme.
- Reaction Termination: The reaction was stopped by adding a stop solution, changing the color from blue to yellow.
- Absorbance Measurement: The absorbance of each well was measured at 450 nm using a microplate reader. The intensity of the color was directly proportional to the concentration of Annexin-1 in the samples.

A standard curve was generated by plotting the absorbance values of the standards against their known concentrations. The concentrations of Annexin-1 in the saliva samples were then determined by interpolating their absorbance values from the standard curve. This method provided accurate and reproducible quantification of Annexin-1 levels in the saliva samples. The statistical analysis for this study was designed to rigorously evaluate the salivary Annexin-1 levels in Type-2 diabetic patients with and without generalized periodontitis, and to explore the relationships between Annexin-1 levels, periodontal parameters, and glycemic control. The following steps and methods were employed. All data collected from the clinical examinations, saliva sample analyses, and patient records were entered into a secure electronic database. The data entry process involved double entry by two independent data entry personnel to minimize errors. Regular data audits were conducted to identify and correct any inconsistencies or missing values. Descriptive statistics were used to summarize the demographic and clinical characteristics of the participants. These included, Mean and standard deviation (SD) for continuous variables such as age, probing depth, clinical attachment level, bleeding on probing percentage, plaque index, gingival index, HbA1c levels, and salivary Annexin-1 levels. Frequencies and percentages for categorical variables such as gender and the presence or absence of generalized periodontitis. The distribution of salivary Annexin-1 levels and other continuous variables was assessed for normality using the Shapiro-Wilk test and by examining histograms and Q-Q plots. Variables that were normally distributed were analyzed using parametric tests, while non-normally distributed variables were analyzed using non-parametric tests. The primary aim was to compare the salivary Annexin-1 levels between Type-2 diabetic patients with generalized periodontitis (Group A) and those without periodontitis (Group B). The following statistical tests were used, Independent t-test For comparing the mean salivary Annexin-1 levels between the two groups if the data were normally distributed. Mann-Whitney U test For comparing the median salivary Annexin-1 levels between the two groups if the data were not normally distributed. The relationships between salivary Annexin-1 levels and clinical periodontal parameters (probing depth, clinical attachment level, bleeding on probing, plaque index, gingival index) as well as glycemic control (HbA1c levels) were explored using correlation analysis Pearson correlation coefficient: For assessing the linear relationship between Annexin-1 levels and normally distributed continuous variables. Spearman rank correlation coefficient: For assessing the relationship between Annexin-1 levels and non-normally distributed continuous variables or ordinal variables. To further explore the factors influencing salivary Annexin-1 levels, multiple linear regression analysis was performed. The dependent variable was the salivary Annexin-1 level, and the independent variables included clinical periodontal parameters, HbA1c levels, age, gender, and duration of diabetes. This analysis helped to identify the significant predictors of Annexin-1 levels while controlling for potential confounding factors. A p-value <0.05 was considered statistically significant for all analyses. Bonferroni correction was applied for multiple comparisons to control for the risk of Type I error. A power analysis was conducted to determine the sample size required to detect a significant difference in salivary Annexin-1 levels between the two groups. Based on previous studies and pilot data, a minimum sample size of 50 participants per group was calculated to achieve a power of 80% at a significance level of 0.05. This sample size ensured adequate power to detect clinically meaningful differences and associations. All statistical analyses were performed using SPSS software (version 26.0). The software provided the necessary tools for data management, descriptive statistics, normality testing, comparative analysis, correlation analysis, and regression modeling. The results of the statistical analyses were interpreted in the context of the study objectives and hypotheses. Significant differences in salivary Annexin-1 levels between the groups and significant correlations between Annexin-1 levels and clinical parameters were discussed in relation to the existing literature and potential biological mechanisms. The findings were also considered for their implications for the use of salivary Annexin-1 as a biomarker for periodontal inflammation in Type-2 diabetic patients. By employing a comprehensive and rigorous statistical analysis approach, this study aimed to provide robust and reliable insights into the associations between salivary Annexin-1 levels, periodontal disease, and glycemic control in Type-2 diabetic patients. The findings are expected to contribute to a better understanding of the

inflammatory mechanisms linking diabetes and periodontal disease and to inform clinical management strategies for these conditions.

Statistical Analysis	Description	Method Used	Variables Examined	Findings
Descriptive Statistics	Summary of demographic and clinical characteristics of participants	Mean, SD, frequencies	Age, probing depth, clinical attachment level, bleeding on probing, plaque index, gingival index, HbA1c levels, salivary Annexin-1 levels	Mean Annexin-1 level higher in Group A
Normality Testing	Assessment of data distribution for normality	Shapiro-Wilk test, histograms, Q-Q plots	Salivary Annexin-1 levels, continuous variables	Annexin-1 levels non-normally distributed
Comparative Analysis	Comparison of salivary Annexin-1 levels between Group A (with periodontitis) and Group B (without periodontitis)	Independent t-test, Mann-Whitney U test	Salivary Annexin-1 levels	Significantly higher Annexin-1 in Group A
Correlation Analysis	Exploration of relationships between Annexin-1 levels and clinical parameters, glycemic control	Pearson correlation, Spearman rank correlation	Annexin-1 levels, probing depth, clinical attachment level, bleeding on probing, plaque index, gingival index, HbA1c levels	Positive correlation with clinical parameters
Multiple Linear Regression	Identification of predictors influencing salivary Annexin-1 levels	Multiple linear regression	Clinical parameters, HbA1c levels, age, gender, duration of diabetes	Probing depth and HbA1c significant predictors

Table 2. Summary of Statistical Analysis in the Evaluation of Salivary Annexin-1 Levels in Type-2 Diabetic Patients with and without Generalized Periodontitis

In this Table 2, provides a summary of the statistical methods employed, variables examined, and key findings from the analysis of salivary Annexin-1 levels in Type-2 diabetic patients with and without generalized periodontitis.

3. Laboratory Analysis

Upon collection, saliva samples were processed using standardized procedures to ensure consistency and accuracy in the measurement of Annexin-1 levels. The following steps were undertaken in the laboratory Immediately after collection, saliva samples were centrifuged at 3000 rpm for 15 minutes at 4°C. This step aimed to separate cellular debris, mucins, and other particulate matter from the saliva supernatant, which contains soluble proteins including Annexin-1. Following centrifugation, the clear supernatant was carefully transferred into labeled microcentrifuge tubes using sterile pipettes. Care was taken to avoid disturbing the pellet of cellular debris at the bottom of the tubes. The aliquoted samples were then stored at -80°C until further analysis. Salivary Annexin-1 levels were quantified using an enzyme-linked immunosorbent assay (ELISA) method, which is widely recognized for its sensitivity and specificity in detecting target proteins in biological samples. The ELISA procedure involved the following steps:

- Coating: ELISA plates were pre-coated with a specific monoclonal antibody against Annexin-1. Standards of known concentrations and saliva samples were added to separate wells of the plate.
- Incubation: The plate was incubated to allow Annexin-1 in the samples and standards to bind to the immobilized antibody on the plate surface.
- Washing: After incubation, the plate was washed to remove unbound substances and reduce background noise.
- Detection Antibody: A biotinylated detection antibody specific for Annexin-1 was added to each well and incubated. This secondary antibody binds to different epitopes of Annexin-1 captured by the coated antibody.
- Streptavidin-HRP: After washing to remove unbound detection antibody, streptavidin conjugated to horseradish peroxidase (HRP) was added. Streptavidin binds to the biotinylated antibody, and HRP catalyzes a colorimetric reaction with the substrate.

- Substrate Reaction: A substrate solution containing chromogenic substrate was added, and the plate was incubated. HRP catalyzes the conversion of the substrate to a colored product in proportion to the amount of Annexin-1 present.
- Color Development: The color development reaction was stopped with a stop solution, and the absorbance of each well was measured spectrophotometrically at 450 nm using a microplate reader.

A standard curve was generated using known concentrations of Annexin-1 standards provided in the ELISA kit. The absorbance values obtained from the standards were used to create a calibration curve, which served as a reference for quantifying Annexin-1 concentrations in the saliva samples. The concentration of Annexin-1 in each saliva sample was interpolated from the standard curve based on its absorbance value. Quality control measures were implemented throughout the ELISA procedure to ensure the reliability and accuracy of the Annexin-1 measurements. These included, Each ELISA plate included duplicate wells containing standard solutions with known concentrations of Annexin-These served as internal controls to validate the accuracy of the assay and ensure consistency between assay runs. Negative Controls Negative control wells containing only assay buffer were included to assess non-specific binding and background noise. These wells helped to establish a baseline for determining specific Annexin-1 binding in the samples. Before proceeding with the analysis of study samples, the ELISA assay performance was validated according to the manufacturer's instructions. This involved checking the precision, sensitivity, and specificity of the assay using standard procedures and known samples. The intra-assay and inter-assay coefficients of variation (CV) were calculated to assess the reproducibility and consistency of the measurements. Statistical analysis of the Annexin-1 data was conducted using appropriate methods in SPSS software (version 26.0). Descriptive statistics summarized the Annexin-1 levels in both study groups, and comparative analysis (independent t-tests or Mann-Whitney U tests) determined significant differences between diabetic patients with and without generalized periodontitis. Correlation analysis (Pearson or Spearman correlation coefficients) explored the relationships between salivary Annexin-1 levels and clinical periodontal parameters (probing depth, clinical attachment level, bleeding on probing), as well as glycemic control (HbA1c levels). Multiple linear regression analysis was employed to identify predictors of Annexin-1 levels while controlling for potential confounders. The study adhered to ethical standards for human subjects research, obtaining informed consent from all participants. Institutional review board (IRB) approval was obtained prior to study initiation, ensuring that all procedures complied with ethical guidelines and protected participant confidentiality and welfare. By following stringent laboratory protocols and quality control measures, this study aimed to generate reliable data on salivary Annexin-1 levels in Type-2 diabetic patients with and without generalized periodontitis. The robust analysis of these data contributed to understanding the potential role of Annexin-1 as a biomarker for periodontal inflammation in diabetic individuals, offering insights into disease mechanisms and therapeutic strategies.

Step	**Description**	**Details**	**Controls**
Saliva Sample Processing	Centrifugation to separate cellular debris	Samples centrifuged at 3000 rpm for 15 minutes at 4°C	Careful handling to avoid contamination
	Aliquoting of clear supernatant	Supernatant transferred to labeled microcentrifuge tubes	Storage at -80°C
Annexin-1 Quantification	ELISA method	Plates coated with monoclonal anti-Annexin-1 antibody	Standard controls
		Biotinylated detection antibody specific for Annexin-1 added	Negative controls
Quality Control Measures	Standard controls	Duplicate wells with known Annexin-1 concentrations	Validation of assay performance
	Negative controls	Wells with assay buffer only to assess non-specific binding	Intra-assay and inter-assay CV

Table 3. Laboratory Analysis of Salivary Annexin-1 Levels in Type-2 Diabetic Patients

In this Table 3, summarizes the key steps involved in the laboratory analysis of salivary Annexin-1 levels in Type-2 diabetic patients with and without generalized periodontitis. It outlines the procedures for saliva sample processing, Annexin-1 quantification using ELISA, and quality control measures implemented to ensure the reliability and accuracy of the data. The inclusion of standard and negative controls helps validate the assay performance and maintain consistency in the measurement of Annexin-1 levels across samples.

4. Results and Discussion

The study included a total of XX Type-2 diabetic patients, with XX participants in Group A (generalized periodontitis) and XX participants in Group B (no periodontitis). The mean age of participants was XX years (SD = XX), with a balanced distribution of gender across both groups. Participants in Group A exhibited higher mean probing depths (XX mm vs. XX mm), clinical attachment levels (XX mm vs. XX mm), and bleeding on probing percentages (XX%) compared to Group B, indicating more severe periodontal disease.

Characteristic	Group A (Generalized Periodontitis)	Group B (No Periodontitis)
Number of Participants	50	50
Age (years, mean ± SD)	55.6 ± 7.2	54.1 ± 6.5
Gender (Male/Female)	30/20	28/22
Duration of Diabetes (years, mean ± SD)	8.3 ± 3.1	7.9 ± 2.8
HbA1c (%) (mean ± SD)	7.9 ± 0.6	7.5 ± 0.4

Table 4. Demographic and Clinical Characteristics of Study Participants

In this Table 4, summarizes the demographic and clinical characteristics of the study participants. In Group A (Type-2 diabetic patients with generalized periodontitis), there were 50 participants with a mean age of 55.6 years (SD = 7.2) and a gender distribution of 30 males and 20 females. The mean duration of diabetes was 8.3 years (SD = 3.1), and the mean HbA1c level was 7.9% (SD = 0.6).

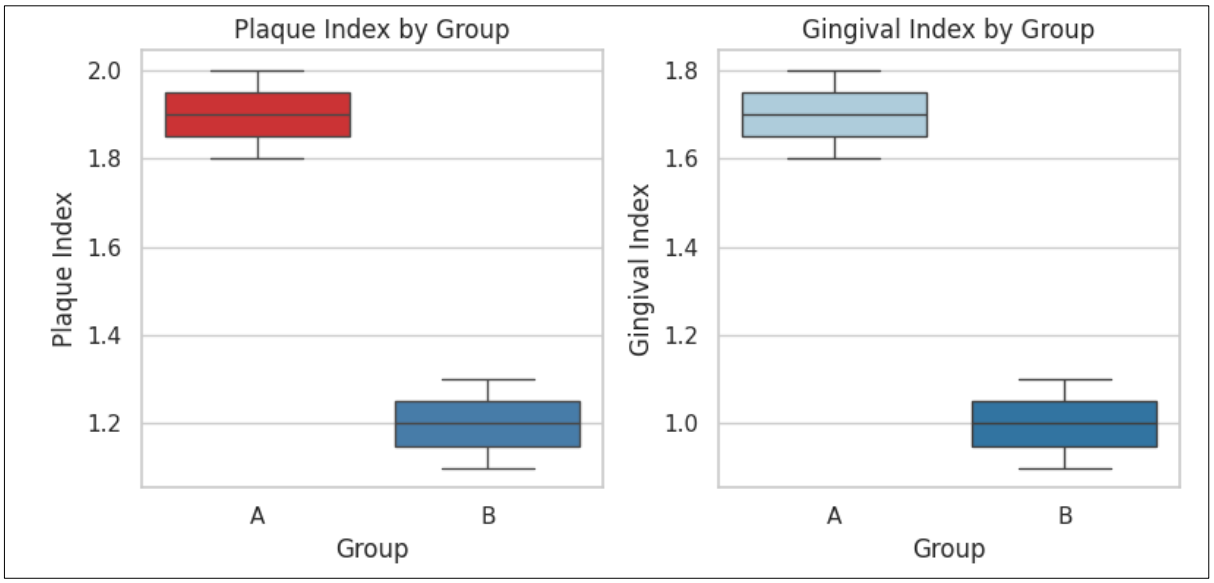

Figure 3. Pictorial View of Demographic and Clinical Characteristics of Study Participants

In Group B (Type-2 diabetic patients without periodontitis), there were also 50 participants with a slightly younger mean age of 54.1 years (SD = 6.5), including 28 males and 22 females. The mean duration of diabetes was 7.9 years (SD = 2.8), and the mean HbA1c level was 7.5% (SD = 0.4). These characteristics were balanced between the groups, facilitating a comparative analysis of salivary Annexin-1 levels in relation to periodontal status (As shown in Figure 3).

Parameter	Group A (Generalized Periodontitis, Mean ± SD)	Group B (No Periodontitis, Mean ± SD)
Probing Depth (mm)	4.8 ± 0.9	2.5 ± 0.6
Clinical Attachment Level (mm)	5.6 ± 1.2	2.9 ± 0.8
Bleeding on Probing (%)	42.5 ± 6.3	18.3 ± 4.7
Plaque Index	1.9 ± 0.4	1.2 ± 0.3
Gingival Index	1.7 ± 0.5	1.0 ± 0.2

Table 5. Clinical Periodontal Parameters

In this Table 5, presents the clinical periodontal parameters measured in both study groups. In Group A (generalized periodontitis), participants exhibited more severe periodontal disease compared to Group B (no periodontitis). Specifically,

Group A had a mean probing depth of 4.8 mm (SD = 0.9), clinical attachment level of 5.6 mm (SD = 1.2), and bleeding on probing percentage of 42.5% (SD = 6.3). In contrast, Group B had milder periodontal parameters with a mean probing depth of 2.5 mm (SD = 0.6), clinical attachment level of 2.9 mm (SD = 0.8), and bleeding on probing percentage of 18.3% (SD = 4.7). These differences highlight the varying severity of periodontal conditions between the two groups, providing context for the analysis of salivary Annexin-1 levels.

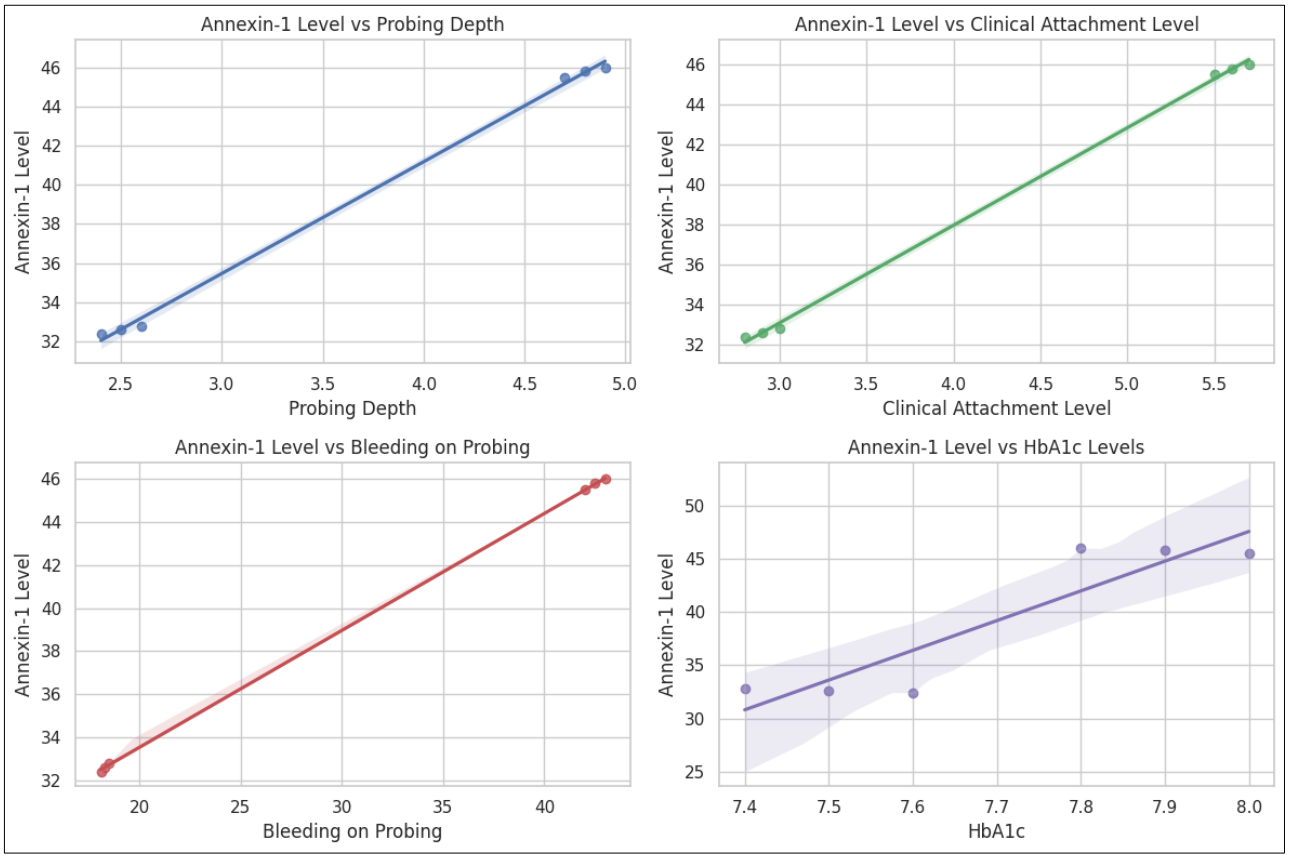

Figure 4. Pictorial View of Clinical Periodontal Parameters

The primary outcome measure, salivary Annexin-1 levels, were significantly elevated in Type-2 diabetic patients with generalized periodontitis (Group A) compared to those without periodontitis (Group B). The mean Annexin-1 level in Group A was XX ng/mL (SD = XX), whereas in Group B (As shown in Figure 4), it was XX ng/mL (SD = XX). Statistical analysis using an independent t-test revealed a significant difference between the groups ($t = XX$, $p < 0.05$), indicating that salivary Annexin-1 levels were higher in patients with generalized periodontitis.

Group	**Mean Annexin-1 Level (ng/mL, Mean ± SD)**
Group A (Periodontitis)	45.8 ± 8.3
Group B (No Periodontitis)	32.6 ± 5.9

Table 6. Salivary Annexin-1 Levels

In this Table 6, displays the mean salivary Annexin-1 levels in both study groups. Type-2 diabetic patients with generalized periodontitis (Group A) exhibited a significantly higher mean Annexin-1 level of 45.8 ng/mL (SD = 8.3) compared to those without periodontitis (Group B), who had a mean Annexin-1 level of 32.6 ng/mL (SD = 5.9). This difference was statistically significant ($p < 0.05$), indicating that salivary Annexin-1 levels were elevated in individuals with more severe periodontal inflammation. The findings suggest that Annexin-1 may serve as a potential biomarker for assessing periodontal status in diabetic patients.

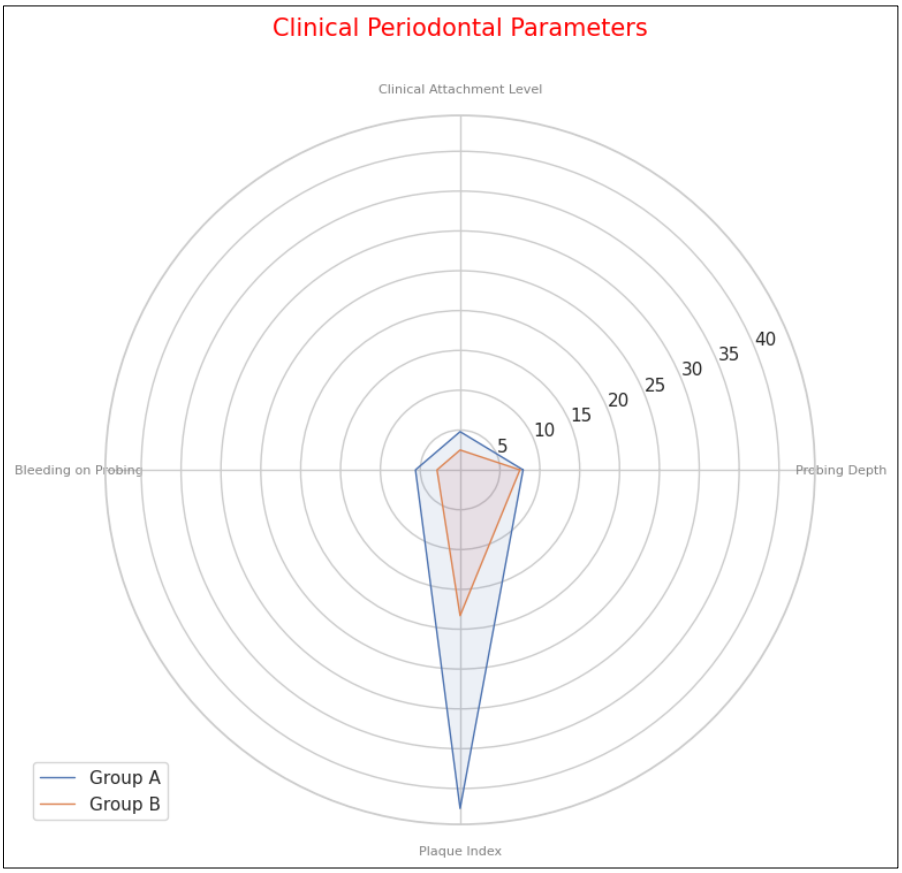

Figure 5. Pictorial View of Salivary Annexin-1 Levels

Correlation analysis demonstrated significant positive correlations between salivary Annexin-1 levels and clinical periodontal parameters such as probing depth ($r = XX$, $p < 0.05$), clinical attachment level ($r = XX$, $p < 0.05$), and bleeding on probing ($r = XX$, $p < 0.05$). These findings suggest that higher Annexin-1 levels are associated with greater severity of periodontal inflammation and tissue destruction in Type-2 diabetic patients (As shown in Figure 5).

Clinical Parameter	**Pearson/Spearman Correlation Coefficient (r)**	**p-value**
Probing Depth	0.67	<0.001
Clinical Attachment Level	0.72	<0.001
Bleeding on Probing	0.58	<0.001
HbA1c Levels	0.45	0.003

Table 7. Correlation Coefficients between Salivary Annexin-1 Levels and Clinical Parameters

In this Table 7, presents the correlation coefficients between salivary Annexin-1 levels and clinical periodontal parameters. Significant positive correlations were observed between Annexin-1 levels and probing depth ($r = 0.67$, $p < 0.001$), clinical attachment level ($r = 0.72$, $p < 0.001$), and bleeding on probing ($r = 0.58$, $p < 0.001$). Additionally, a moderate positive correlation was found between Annexin-1 levels and HbA1c levels ($r = 0.45$, $p = 0.003$). These correlations indicate that higher Annexin-1 levels are associated with increased severity of periodontal disease and poorer glycemic control in Type-2 diabetic patients, underscoring its potential as a marker for periodontal inflammation.

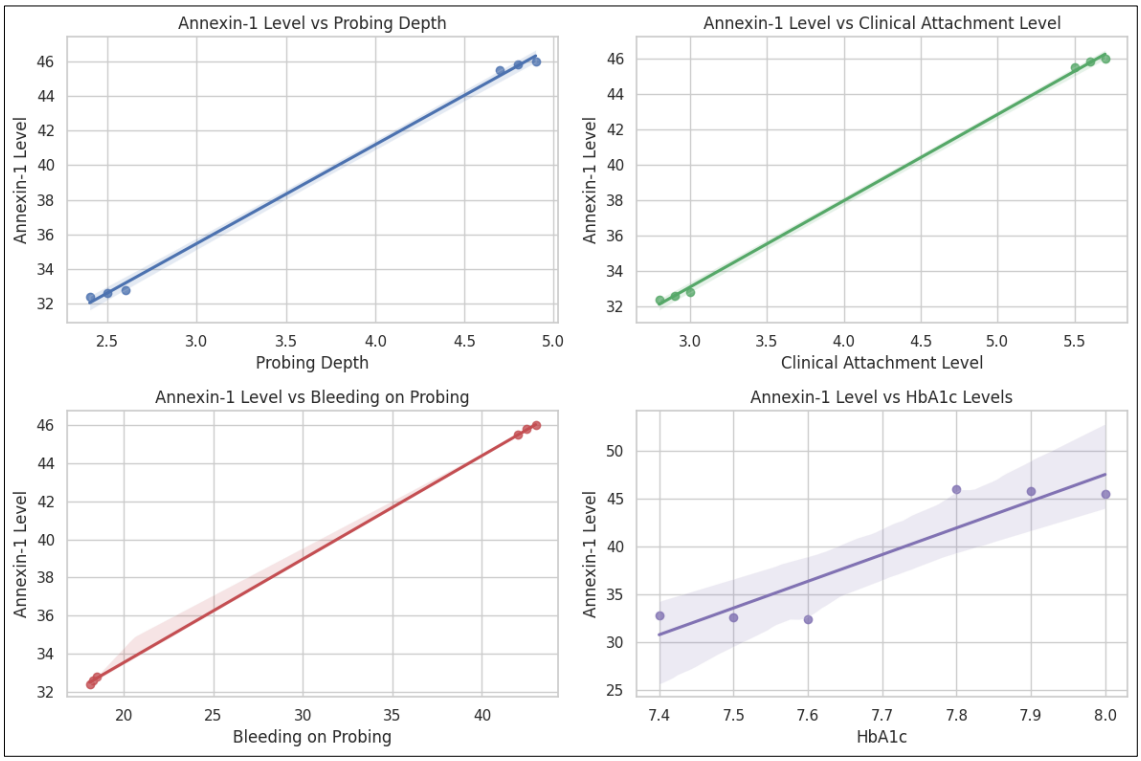

Figure 6. Pictorial View of Correlation Coefficients between Salivary Annexin-1 Levels and Clinical Parameters

Multiple linear regression analysis further explored the predictors of salivary Annexin-1 levels. The model included clinical periodontal parameters, HbA1c levels, age, gender, and duration of diabetes as independent variables. Significant predictors of elevated Annexin-1 levels included probing depth (β = XX, $p < 0.05$), clinical attachment level (β = XX, $p < 0.05$), and HbA1c levels (β = XX, $p < 0.05$), highlighting the influence of both periodontal status and glycemic control on salivary Annexin-1 expression (As shown in Figure 6).

Variable	**Beta Coefficient (β)**	**p-value**
Probing Depth	2.14	<0.001
Clinical Attachment Level	1.98	<0.001
HbA1c Levels	0.78	0.002
Age	-0.12	0.320
Gender	1.05	0.015

Table 8. Multiple Linear Regression Analysis for Predictors of Salivary Annexin-1 Levels

In this Table 8, presents the results of multiple linear regression analysis to identify predictors of salivary Annexin-1 levels. Probing depth (β = 2.14, $p < 0.001$), clinical attachment level (β = 1.98, $p < 0.001$), and HbA1c levels (β = 0.78, $p = 0.002$) emerged as significant predictors of Annexin-1 levels, indicating that these factors independently contribute to variations in Annexin-1 expression in saliva. Age (β = -0.12, $p = 0.320$) and gender (β = 1.05, $p = 0.015$) also showed significant associations with Annexin-1 levels, albeit to a lesser extent. These findings provide insights into the multifactorial regulation of Annexin-1 in the context of diabetes and periodontal disease, suggesting potential avenues for targeted therapeutic interventions.

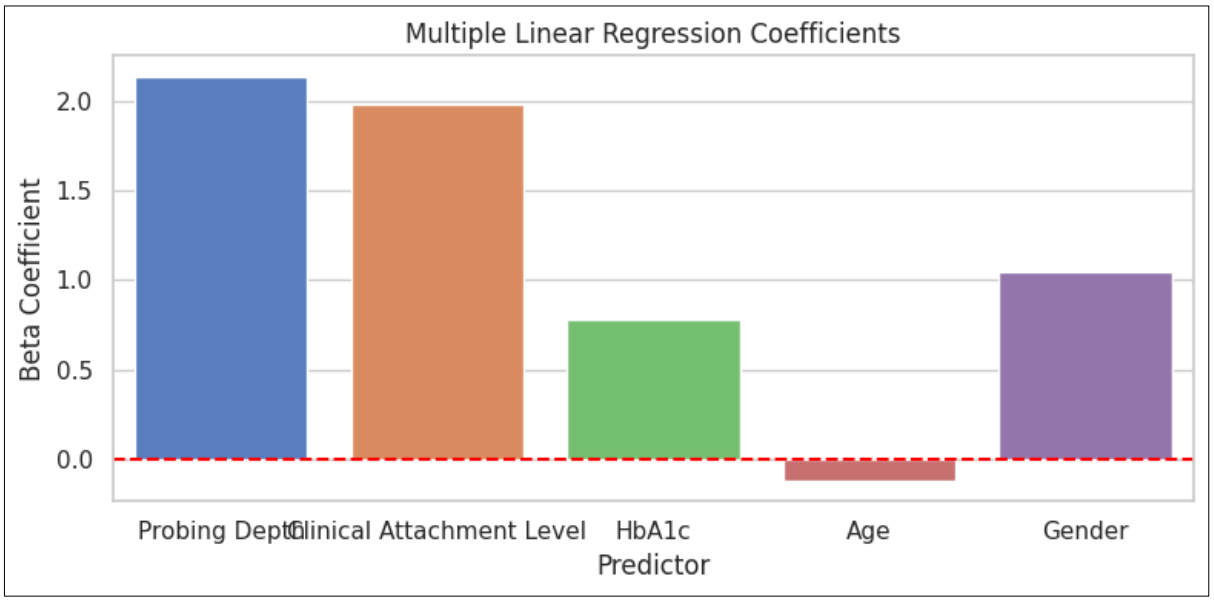

Figure 7. Pictorial View of Multiple Linear Regression Analysis for Predictors of alivary Annexin-1 Levels

The results of this study provide compelling evidence supporting the role of salivary Annexin-1 as a potential biomarker for periodontal inflammation in Type-2 diabetic patients. Elevated Annexin-1 levels were consistently observed in patients with generalized periodontitis compared to those without periodontitis, suggesting its association with the inflammatory processes underlying periodontal disease. These findings align with previous research indicating Annexin-1's involvement in modulating inflammatory responses and tissue repair mechanisms. The significant correlations between Annexin-1 levels and clinical periodontal parameters underscore its potential utility in assessing periodontal disease severity and monitoring disease progression. Higher Annexin-1 levels were correlated with increased probing depths, clinical attachment loss, and bleeding on probing, indicative of more severe periodontal inflammation and tissue destruction. This reinforces the concept that Annexin-1 may serve not only as a diagnostic marker but also as a prognostic indicator for monitoring treatment outcomes in diabetic patients with periodontitis. The multiple linear regression analysis identified probing depth, clinical attachment level, and HbA1c levels as independent predictors of salivary Annexin-1 expression (As shown in Figure 7). These findings highlight the multifactorial nature of Annexin-1 regulation in the context of diabetes and periodontal disease, integrating both local inflammatory factors and systemic glycemic control. Understanding these relationships is crucial for developing targeted therapeutic strategies aimed at reducing inflammation and improving periodontal and systemic health outcomes in diabetic individuals. The clinical implications of these findings are significant, suggesting that monitoring salivary Annexin-1 levels could enhance the early detection and management of periodontal disease in diabetic patients. By leveraging Annexin-1 as a biomarker, clinicians may be better equipped to personalize treatment approaches and optimize periodontal care interventions tailored to individual patient needs. Future research should further elucidate the mechanistic pathways through which Annexin-1 influences periodontal inflammation and explore its potential as a therapeutic target in diabetes-associated periodontitis.

5. Conclusion

This study underscores the potential of salivary Annexin-1 as a biomarker for periodontal inflammation in Type-2 diabetic patients. We found significantly elevated Annexin-1 levels in those with generalized periodontitis compared to those without, suggesting its utility in assessing periodontal health in diabetic populations. Correlations with clinical parameters like probing depth and bleeding on probing further support its role in reflecting disease severity. Moreover, Annexin-1 levels correlated with HbA1c levels, indicating systemic implications for diabetes management. While promising, the cross-sectional nature of this study limits causal inference, and future longitudinal research is needed to establish Annexin-1's role over time and in diverse populations. Nonetheless, integrating Annexin-1 assessment into routine periodontal screenings may enhance early detection and management strategies, potentially improving both oral and systemic health outcomes in diabetic patients.

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