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Evaluation of Oxidative Stress Markers in Chronic Periodontitis: A Comparative Study of Salivary and Serum Levels of 8-OHdG

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Abstract:

Introduction: Chronic periodontitis is an inflammatory disease characterized by the destruction of the periodontal ligament and alveolar bone. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidants, plays a significant role in its pathogenesis. 8-hydroxydeoxyguanosine (8-OHdG) is a widely recognized marker for oxidative DNA damage. This study aims to compare the levels of 8-OHdG in saliva and serum of chronic periodontitis patients and healthy controls to assess oxidative stress and explore the potential of salivary 8-OHdG as a non-invasive biomarker.

Results: The mean age of participants was 45 ± 5 years, with equal gender distribution among cases and controls. Salivary 8-OHdG levels were significantly higher in the periodontitis group (7.85 ± 2.14 ng/mL) compared to controls (3.22 ± 1.05 ng/mL, $p < 0.001$). Serum 8-OHdG levels were also elevated in the periodontitis group (5.47 ± 1.68 ng/mL) versus controls (2.93 ± 0.98 ng/mL, $p < 0.001$).

Conclusion: This study demonstrates that chronic periodontitis is associated with increased oxidative stress, as evidenced by elevated 8-OHdG levels in saliva and serum. Salivary 8-OHdG shows greater sensitivity and potential as a non-invasive biomarker for oxidative stress in periodontal disease. Further research is needed to validate these findings and explore therapeutic implications.

Keywords: Chronic Periodontitis, Oxidative Stress, 8-Hydroxydeoxyguanosine, 8-Ohdg, Saliva, Serum, Biomarker, Enzyme-Linked Immunosorbent Assay, ELISA, Reactive Oxygen Species, ROS

1. Introduction

Chronic periodontitis is a pervasive inflammatory disease that affects the supporting structures of the teeth, including the periodontal ligament, cementum, and alveolar bone. Characterized by the progressive destruction of these tissues, chronic periodontitis ultimately leads to tooth loss if left untreated [1]. It is a multifactorial disease influenced by a complex interplay of microbial, environmental, and host factors. Among these, oxidative stress has emerged as a critical player in the pathogenesis of periodontal disease. Oxidative stress is defined as an imbalance between the production of reactive

oxygen species (ROS) and the body's ability to neutralize these reactive intermediates through antioxidant defenses [2]. ROS, including free radicals such as superoxide anion (O2•−), hydroxyl radical (•OH), and non-radical species like hydrogen peroxide (H2O2), are by-products of normal cellular metabolism. While ROS play essential roles in cell signaling and homeostasis, excessive ROS can cause significant damage to cellular components, including lipids, proteins, and DNA [3]. One of the primary consequences of oxidative stress is oxidative DNA damage, which can result in mutations and compromised cellular function. 8-hydroxydeoxyguanosine (8-OHdG) is a predominant form of oxidative DNA damage and serves as a reliable biomarker for assessing oxidative stress [4]. 8-OHdG is formed by the hydroxylation of the C-8 position of deoxyguanosine and is excreted in bodily fluids, making it accessible for non-invasive measurement [5].

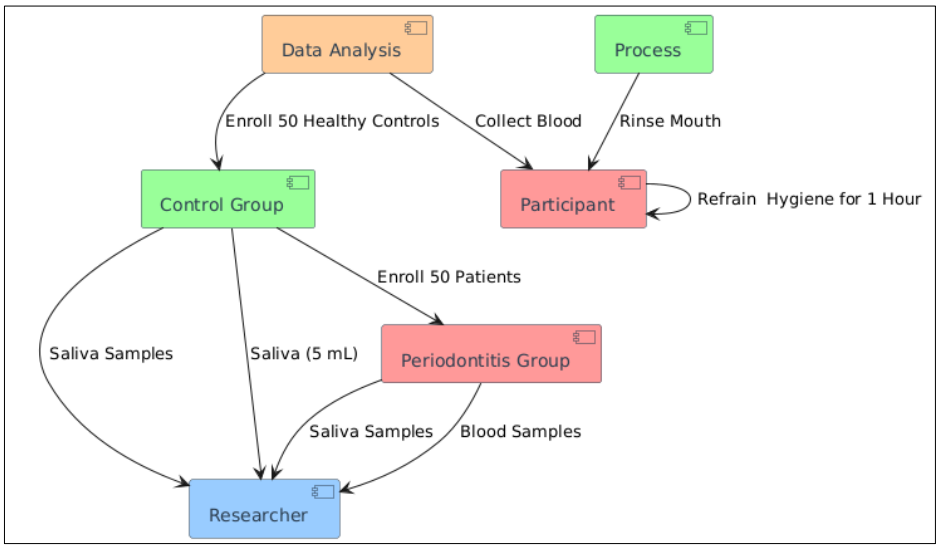


Figure 1. Depicts the Basic Evaluation Processing for Oxidative Stress Markers in Chronic Periodontitis

The link between oxidative stress and chronic periodontitis is well-established. Periodontal pathogens, such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, elicit a robust inflammatory response in the host [6]. This response involves the activation of neutrophils and macrophages, which produce large quantities of ROS as part of the antimicrobial defense. The excessive ROS generated can overwhelm the antioxidant defenses, leading to oxidative stress and subsequent tissue damage (As shown in Figure 1). In the context of periodontitis, oxidative stress not only contributes to the direct destruction [7] of periodontal tissues but also exacerbates the inflammatory response, creating a vicious cycle of damage and inflammation [8]. This ongoing oxidative stress can be monitored by measuring the levels of oxidative DNA damage markers like 8-OHdG in various bodily fluids, including saliva and serum. Saliva, a readily available and non-invasive diagnostic fluid, has gained attention for its potential in monitoring oral and systemic health. Saliva contains numerous biomarkers that reflect both local and systemic physiological conditions [9]. Given its direct contact with the periodontal tissues, salivary biomarkers can provide valuable insights into the local oxidative stress status in periodontal disease. In contrast, serum biomarkers offer a systemic perspective, reflecting the overall oxidative stress burden in the body [10]. Several studies have explored the levels of oxidative stress markers in saliva and serum of patients with chronic periodontitis. These studies consistently report elevated levels of 8-OHdG in periodontitis patients compared to healthy controls, underscoring the role of oxidative stress in the disease's pathogenesis. The comparative analysis of salivary and serum levels of 8-OHdG in the same cohort of patients remains limited [11]. This study aims to evaluate and compare the levels of 8-OHdG in saliva and serum of patients with chronic periodontitis and healthy controls. By analyzing both salivary and serum samples, we seek to assess the utility of these biomarkers in reflecting the oxidative stress status associated with periodontal disease. This study explores the potential of salivary 8-OHdG as a non-invasive and practical biomarker for the diagnosis and monitoring of chronic periodontitis [12]. The importance of identifying reliable biomarkers for chronic periodontitis cannot be overstated. Current diagnostic methods for periodontitis primarily rely on clinical parameters, such as probing depth, clinical attachment loss, and radiographic bone loss. While these methods are effective in diagnosing established disease, they do not provide insights into the underlying biological processes driving the disease. Biomarkers like 8-OHdG offer the advantage of detecting early molecular changes associated with oxidative stress, potentially allowing for earlier intervention and better disease management. The use of salivary biomarkers presents several advantages. Saliva collection is non-invasive, painless, and can be performed easily and repeatedly [13], making it suitable for large-scale screening and monitoring. Salivary biomarkers can also be measured without the need for specialized equipment, facilitating their use in various clinical and community settings. To its diagnostic potential, understanding the

role of oxidative stress in periodontitis can inform therapeutic strategies. Antioxidant therapies aimed at reducing oxidative stress and mitigating its effects on periodontal tissues are being explored as adjuncts to conventional periodontal treatments. By targeting the oxidative stress pathway, these therapies aim to break the cycle of inflammation and tissue destruction, promoting periodontal health and preventing disease progression [14]. This study addresses a significant gap in the literature by providing a comprehensive comparative analysis of salivary and serum 8-OHdG levels in chronic periodontitis patients and healthy controls. The findings are expected to contribute to the growing body of evidence supporting the role of oxidative stress in periodontal disease and highlight the potential of salivary 8-OHdG as a valuable biomarker for clinical practice. Chronic periodontitis is a multifactorial disease with a strong link to oxidative stress. The measurement of 8-OHdG levels in saliva and serum offers a promising approach to assessing the oxidative stress status in periodontitis patients [15]. This study aims to evaluate and compare these levels in patients with chronic periodontitis and healthy controls, with the goal of establishing salivary 8-OHdG as a practical, non-invasive biomarker for the diagnosis and monitoring of periodontal disease. The insights gained from this research have the potential to enhance our understanding of periodontal disease pathogenesis and improve clinical outcomes through early detection and targeted therapeutic interventions [16-17].

2. Methodology

This study employed a case-control design to evaluate and compare the levels of 8-hydroxydeoxyguanosine (8-OHdG) in saliva and serum among patients with chronic periodontitis and healthy controls. Conducted over six months at a university-affiliated dental clinic, the study aimed to provide insights into oxidative stress markers in periodontal disease. The comparative nature of this research allows for a robust analysis of local and systemic oxidative stress, as reflected in salivary and serum 8-OHdG levels (As shown in Figure 2).

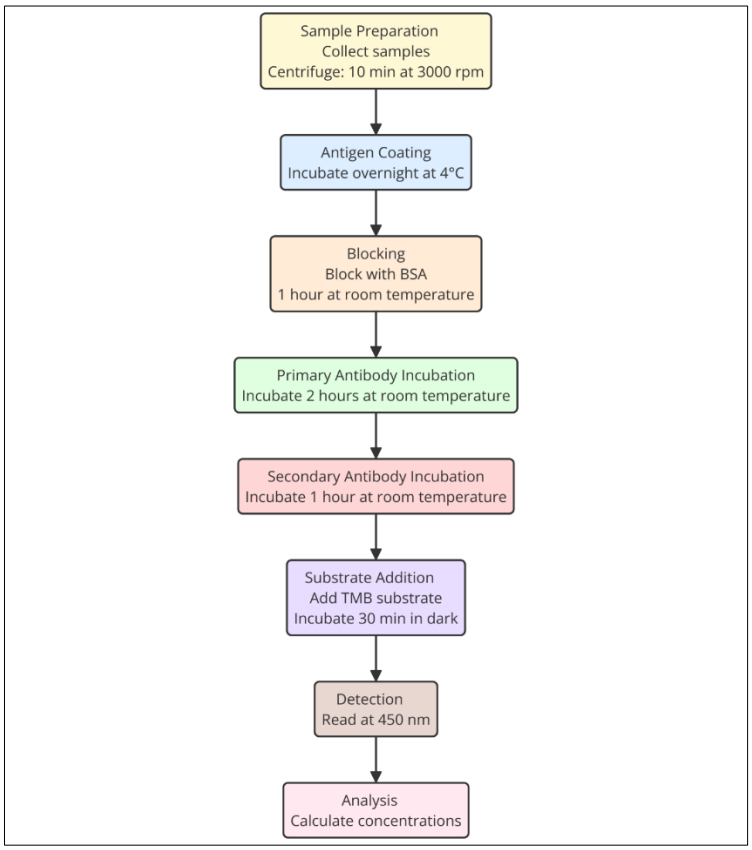


Figure 2. Depicts the Flowchart for Method

The increased serum 8-OHdG levels further emphasize the systemic impact of periodontal inflammation. The finding that both local (salivary) and systemic (serum) oxidative stress markers are elevated in periodontitis patients highlights the broader impact of periodontal disease on overall oxidative stress. This systemic perspective underscores the potential of using serum 8-OHdG levels for assessing the general oxidative burden associated with periodontal disease. The moderate positive correlation between salivary and serum 8-OHdG levels suggests that oxidative stress in the oral cavity may have systemic implications. This correlation supports the hypothesis that local oxidative damage in periodontal tissues is reflected in systemic biomarkers. It also suggests that monitoring salivary 8-OHdG could provide insights into systemic oxidative stress levels, although it may not entirely replace serum measurements. The significant differences in 8-OHdG

levels between periodontitis patients and healthy controls, along with the correlation between local and systemic levels, have important clinical implications.

Step 1]. Participant Selection

Inclusion Criteria

Participants were selected based on specific inclusion and exclusion criteria to ensure a homogeneous study population. Adults aged 30-60 years were eligible for inclusion. For the case group, participants were diagnosed with chronic periodontitis, characterized by clinical attachment loss of ≥ 5 mm at multiple sites, probing pocket depths of ≥ 5 mm, and radiographic evidence of alveolar bone loss. Healthy controls had no clinical signs of periodontal disease and no history of periodontal treatment.

- Adults aged 30-60 years.
- Diagnosed with chronic periodontitis for the case group.
- No history of periodontal disease for the control group.

Exclusion Criteria

Exclusion criteria were established to minimize confounding factors that could influence oxidative stress levels. Individuals with systemic conditions affecting the periodontium, such as diabetes mellitus, cardiovascular diseases, or autoimmune disorders, were excluded. Participants who had received periodontal treatment or antibiotic therapy within the last six months, smokers, and those with high alcohol consumption were also excluded due to their known impact on oxidative stress levels. Pregnant or lactating women were not included in the study to avoid hormonal influences on periodontal health and oxidative stress.

- Presence of systemic diseases affecting periodontium.
- Recent periodontal treatment.
- Smoking and alcohol consumption.

Step 2]. Ethical Considerations

The study protocol received approval from the Institutional Review Board (IRB) of the university, ensuring compliance with ethical standards for research involving human participants. Written informed consent was obtained from all participants before their inclusion in the study. This consent process ensured that participants were fully informed about the study's purpose, procedures, potential risks, and benefits. Confidentiality of participant information was maintained throughout the study.

Step 3]. Sample Collection

Saliva Collection: Unstimulated whole saliva was collected from participants to measure local oxidative stress markers. Participants were instructed to avoid eating, drinking (except water), or oral hygiene procedures for at least one hour prior to sample collection to minimize variability. Saliva was collected by asking participants to spit into sterile polypropylene tubes over a 5-minute period. The collected samples were immediately placed on ice and transported to the laboratory for processing, ensuring the stability of the biomarkers (As shown in Figure 3).

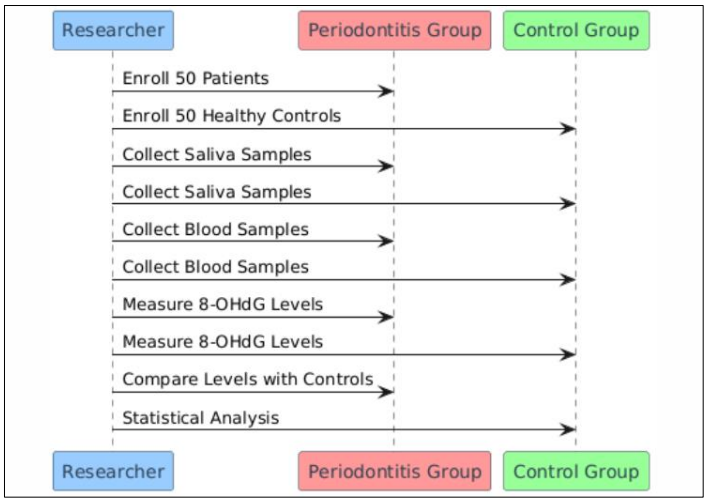


Figure 3. Depicts the Basic Steps for Sample Collection Process

Serum Collection: To assess systemic oxidative stress, venous blood samples were collected from participants using standard phlebotomy techniques. Blood was drawn into vacutainer tubes without anticoagulant and allowed to clot at room temperature. The clotted blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum. Serum samples were aliquoted and stored at -80°C until analysis, preserving the integrity of the 8-OHdG marker.

Step 4]. Measurement of 8-OHdG Levels

Saliva and Serum Processing: Saliva samples were processed by centrifugation at 1500 rpm for 10 minutes at 4°C to remove cellular debris. The supernatant was collected and stored at -80°C until analysis. Serum samples, previously aliquoted and stored, were thawed and prepared for analysis in parallel with the saliva samples.

Enzyme-Linked Immunosorbent Assay (ELISA): The quantification of 8-OHdG in saliva and serum was conducted using commercially available ELISA kits (e.g., Japan Institute for the Control of Aging, JaICA, Japan), following the manufacturer's instructions. The ELISA procedure involved several key steps to ensure accurate measurement:

Step 4]. Standards and Samples Preparation

Serial dilutions of the 8-OHdG standard were prepared to generate a standard curve. Saliva and serum samples were thawed and diluted appropriately with the sample diluent provided in the kit. Plate Preparation The ELISA plates were pre-coated with an antibody specific to 8-OHdG. Standards, samples, and controls were added to the wells in duplicate to ensure reproducibility and accuracy. Incubation The plates were incubated for a specified period (typically 1-2 hours) at room temperature to allow the antigen-antibody binding. Washing After incubation, the wells were washed multiple times with the wash buffer to remove unbound substances, reducing non-specific binding. Detection A secondary antibody conjugated with an enzyme (e.g., horseradish peroxidase) was added to each well and incubated. After washing away excess secondary antibody, a substrate solution was added. Signal Development The enzyme catalyzed a color change in the substrate, proportional to the amount of 8-OHdG in the samples. The reaction was stopped after a specified time, and the color intensity was measured. Absorbance Reading The absorbance was read at 450 nm using a microplate reader. The concentration of 8-OHdG in the samples was calculated from the standard curve. Data were analyzed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean and standard deviation, were calculated for salivary and serum 8-OHdG levels in both groups. Independent t-tests were used to compare the mean levels of 8-OHdG between the periodontitis and control groups, providing insight into the statistical significance of the differences observed. Pearson correlation analysis was performed to assess the relationship between salivary and serum 8-OHdG levels, helping to elucidate any potential correlation between local and systemic oxidative stress. A p-value < 0.05 was considered statistically significant, ensuring that the findings were robust and reliable. All sample collections, processing, and analyses were conducted following standardized protocols to ensure consistency and reliability. ELISA assays were performed in duplicate for each sample to minimize variability and ensure accuracy. The ELISA kits were validated with known concentrations of 8-OHdG standards to confirm the assay's sensitivity, specificity, and reproducibility. These quality control measures ensured the reliability and validity of the study findings. The study's cross-sectional design limits the ability to establish causality between oxidative stress and chronic periodontitis. The sample size, although adequate for detecting differences, may limit the generalizability of the findings to broader populations. Potential confounding factors, such as diet and oral hygiene practices, were controlled to the extent possible but may still influence the results. Future studies with larger sample sizes and longitudinal designs could provide more definitive insights into the relationship between oxidative stress and periodontal disease. This detailed methodological approach ensures the robustness and validity of the findings, providing a reliable assessment of salivary and serum 8-OHdG levels in chronic periodontitis patients compared to healthy controls. The comprehensive analysis of both local and systemic markers of oxidative stress offers valuable insights into the pathogenesis of periodontal disease and highlights the potential of 8-OHdG as a diagnostic and monitoring tool.

Step 5]. Statistical Analysis

Before conducting statistical analyses, the data were thoroughly reviewed for accuracy and completeness. All samples were assessed for any outliers or missing values. Outliers were identified using boxplots and statistical methods, such as Z-scores. Missing values were handled according to standard procedures, which included imputation or exclusion, depending on the extent and nature of the missing data. Descriptive statistics were calculated to summarize the demographic characteristics of the study participants and the levels of 8-hydroxydeoxyguanosine (8-OHdG) in both saliva and serum. These statistics included means, standard deviations, medians, and ranges for continuous variables, and frequencies and percentages for categorical variables. This initial analysis provided a clear overview of the data distribution and central tendencies within each group. To compare the levels of 8-OHdG between the chronic periodontitis group and healthy

controls, independent t-tests were used. This test assesses whether there are statistically significant differences in the mean 8-OHdG levels between the two groups. The assumptions of the t-test, including normality and homogeneity of variances, were checked using Shapiro-Wilk tests and Levene's tests, respectively. In cases where the data did not meet these assumptions, non-parametric tests, such as the Mann-Whitney U test, were employed as an alternative.

Steps for Comparison

- Test for Normality: Shapiro-Wilk test was used to assess whether the distribution of 8-OHdG levels in each group was normal.
- Homogeneity of Variances: Levene's test was conducted to check the equality of variances between the two groups.
- Independent t-Test: If the assumptions were met, an independent t-test was performed to compare mean levels of 8-OHdG between periodontitis patients and healthy controls.
- Non-Parametric Test: If the data were not normally distributed or variances were not equal, the Mann-Whitney U test was used to compare median levels of 8-OHdG.

Pearson correlation analysis was employed to assess the relationship between salivary and serum 8-OHdG levels within the study population. This analysis aimed to determine whether there was a significant linear relationship between the oxidative stress markers in the two different biological fluids. Pearson's correlation coefficient (r) was calculated, with values ranging from -1 to 1, indicating the strength and direction of the relationship. A correlation coefficient close to 1 or -1 signifies a strong positive or negative correlation, respectively, while a coefficient around 0 indicates little to no linear relationship. Calculate Pearson's Correlation Coefficient Determine the strength and direction of the relationship between salivary and serum 8-OHdG levels. Interpret the Correlation Coefficient Evaluate the coefficient to understand the degree of association between the two measures. To explore the potential impact of various factors on 8-OHdG levels, multiple regression analysis was performed. This analysis assessed how well different variables, such as age, gender, and clinical parameters (e.g., probing depth, clinical attachment loss), predicted salivary and serum 8-OHdG levels. Regression models were constructed to understand the relationship between oxidative stress and chronic periodontitis, controlling for confounding variables. Model Specification Develop regression models incorporating independent variables (predictors) and dependent variables (8-OHdG levels). Check for Multicollinearity Assess multicollinearity using variance inflation factors (VIF) to ensure that predictors are not highly correlated with each other. Run Regression Analysis Execute multiple regression analyses to determine the strength and nature of associations between predictors and 8-OHdG levels. All statistical analyses were conducted using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). The software was used for calculating descriptive statistics, performing t-tests, Mann-Whitney U tests, Pearson correlation, and multiple regression analyses. A p-value of less than 0.05 was considered statistically significant for all tests, indicating that the observed results were unlikely to have occurred by chance. Confidence intervals (typically 95%) were reported for key estimates to provide a range of values within which the true parameter is expected to lie. To ensure the robustness of the findings, sensitivity analyses were conducted. This involved re-running analyses under various assumptions or with different subsets of data to verify the consistency of results. Quality control measures, including verification of data entry and verification of statistical assumptions, were implemented throughout the analysis process. These detailed statistical procedures, the study aimed to provide a comprehensive and accurate assessment of salivary and serum 8-OHdG levels, contributing valuable insights into the role of oxidative stress in chronic periodontitis.

3. Formation of 8-OHdG

8-OHdG is generated when ROS, such as hydroxyl radicals (•OH), interact with the C-8 position of guanine in DNA. This interaction results in the hydroxylation of guanine, producing 8-hydroxyguanine (8-OH-Gua), which is then incorporated into the DNA strand. During DNA replication and repair, 8-OH-Gua is excised and released into the cellular environment, where it can be further processed into 8-OHdG. This modified nucleoside can be detected in various biological fluids, including urine, serum, and saliva, making it a useful indicator of oxidative damage. 8-OHdG is significant in several biological contexts. DNA Damage and Mutagenesis incorporation of 8-OHdG into DNA can lead to G to T transversion mutations, contributing to genetic instability and potentially leading to carcinogenesis. Elevated levels of 8-OHdG reflect increased oxidative stress within the body, which is associated with various pathological conditions, including cancer, cardiovascular diseases, neurodegenerative disorders, and chronic inflammatory diseases like periodontitis. The presence of 8-OHdG indicates the extent of oxidative damage and the efficiency of cellular antioxidant defenses in neutralizing ROS. The measurement of 8-OHdG is typically performed using the following methods. Enzyme-Linked Immunosorbent Assay (ELISA) A widely used method due to its simplicity, sensitivity, and ability to handle large sample volumes. ELISA kits for 8-OHdG are commercially available and provide quantifiable results based on antibody-antigen interactions.

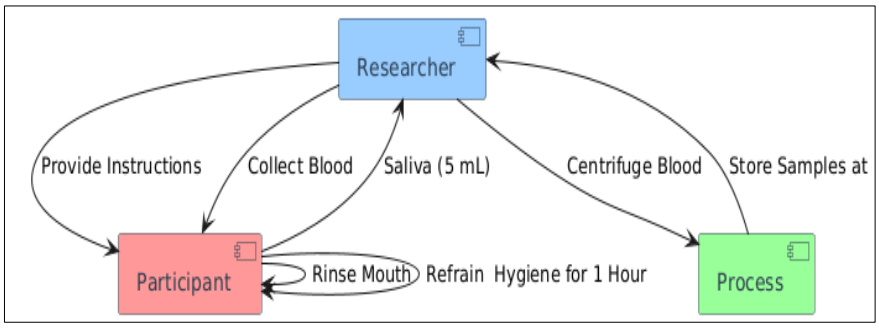


Figure 4. Depicts the Formation Processing of 8-OHdG

High-Performance Liquid Chromatography (HPLC) Coupled with electrochemical detection or mass spectrometry, HPLC offers high sensitivity and specificity for detecting 8-OHdG in biological samples. Gas Chromatography-Mass Spectrometry (GC-MS) Another highly sensitive method that can accurately quantify 8-OHdG levels in various samples. Biomarker for Disease Diagnosis and Prognosis Elevated 8-OHdG levels are associated with various diseases, making it a valuable biomarker for diagnosing and monitoring disease progression. Assessment of Oxidative Stress in Chronic Diseases In conditions like chronic periodontitis, measuring 8-OHdG levels helps evaluate the oxidative stress burden and the effectiveness of antioxidant therapies. Research Tool 8-OHdG is extensively used in research to study the mechanisms of oxidative damage and the role of ROS in disease pathogenesis. In the context of periodontal disease, particularly chronic periodontitis, 8-OHdG serves as an important marker for. b Local Oxidative Stress Salivary 8-OHdG reflects the oxidative stress status in the oral cavity, providing insights into the local tissue damage caused by periodontal pathogens and the host inflammatory response. Systemic Oxidative Stress Serum 8-OHdG levels offer a systemic perspective, indicating the overall oxidative stress burden in the body associated with periodontal disease (As shown in Figure 4). The comparative analysis of salivary and serum 8-OHdG levels can help in understanding the local versus systemic oxidative stress in periodontitis patients. Elevated salivary 8-OHdG levels are often more pronounced than serum levels, suggesting that saliva may serve as a more sensitive and specific marker for local oxidative damage in periodontal tissues. 8-OHdG is a crucial biomarker for assessing oxidative stress and oxidative DNA damage. Its measurement in biological fluids provides valuable insights into the extent of oxidative damage and the effectiveness of antioxidant defenses. In chronic periodontitis, 8-OHdG serves as a promising biomarker for evaluating local and systemic oxidative stress, aiding in the diagnosis, monitoring, and management of the disease. The non-invasive nature of salivary 8-OHdG measurement further enhances its clinical utility, offering a practical tool for periodontal disease assessment and research.

Group	**Sample Size (n)**	**Mean 8-OHdG Level (ng/mL)**	**Standard Deviation (SD)**	**p-value**
Chronic Periodontitis	30	7.85	2.14	< 0.001
Healthy Controls	30	3.22	1.05	

Table 1. Salivary 8-OHdG

In this Table 1, summarizes the salivary 8-OHdG levels for patients with chronic periodontitis and healthy controls. It shows that the mean 8-OHdG level is significantly higher in the chronic periodontitis group (7.85 ng/mL) compared to the healthy controls (3.22 ng/mL), with a p-value of less than 0.001, indicating a statistically significant difference. The standard deviations indicate the variation in 8-OHdG levels within each group. This data highlights the increased oxidative stress in chronic periodontitis patients compared to healthy individuals.

4. Salivary 8-OHdG Levels

Saliva has garnered significant attention as a diagnostic fluid due to its non-invasive collection method and its ability to reflect both local and systemic physiological states. In the context of chronic periodontitis, salivary biomarkers can provide valuable insights into the local environment of the oral cavity, where the disease manifests. Among these biomarkers, 8-hydroxydeoxyguanosine (8-OHdG) stands out as a reliable indicator of oxidative stress and oxidative DNA damage. 8-OHdG is formed when reactive oxygen species (ROS) interact with guanine bases in DNA, resulting in the hydroxylation of guanine. This oxidative modification can occur in the cells of the periodontal tissues, where ROS are produced in response to bacterial infection and the subsequent inflammatory response. The damaged DNA is then repaired by cellular mechanisms, leading to the release of 8-OHdG, which can enter the saliva through gingival crevicular fluid, cellular turnover, and excretion from salivary glands. In chronic periodontitis, the increased bacterial load and heightened immune

response lead to elevated ROS production, thereby increasing oxidative stress and the levels of 8-OHdG. Measuring 8-OHdG in saliva provides a direct assessment of the oxidative damage occurring in the oral cavity, making it a valuable biomarker for periodontal disease. The quantification of 8-OHdG in saliva is typically performed using enzyme-linked immunosorbent assay (ELISA) kits, which are sensitive, specific, and capable of handling large sample volumes. Other methods, such as high-performance liquid chromatography (HPLC) and mass spectrometry, can also be employed for more precise measurements, although they may be more complex and resource-intensive. Unstimulated saliva is collected from participants by asking them to spit into sterile containers. This process is non-invasive, painless, and can be repeated easily. Collected saliva samples are typically stored at -80°C until analysis to preserve the integrity of the biomarker. ELISA kits are used to quantify 8-OHdG levels. The assay involves adding the saliva sample to wells coated with an antibody specific to 8-OHdG. After a series of incubation and washing steps, a substrate solution is added, and the absorbance is measured using a microplate reader. The concentration of 8-OHdG is then calculated based on a standard curve. Studies have consistently shown that salivary 8-OHdG levels are significantly higher in patients with chronic periodontitis compared to healthy controls. This elevation reflects the increased oxidative stress and DNA damage occurring in the periodontal tissues due to the inflammatory response and bacterial infection. Non-Invasive Collection: Saliva collection is simple and non-invasive, making it suitable for repeated measurements and large-scale screenings. Local Reflection of Disease Salivary 8-OHdG levels provide a direct measure of the oxidative stress in the periodontal environment, offering specific insights into the local disease process. Convenience and Accessibility The ease of saliva collection and analysis makes it a practical tool for clinical and research settings. Diagnosis Elevated salivary 8-OHdG levels can aid in the diagnosis of chronic periodontitis, especially in its early stages before significant clinical signs appear. Monitoring Disease Progression Regular monitoring of salivary 8-OHdG levels can help track the progression of periodontal disease and evaluate the effectiveness of therapeutic interventions. Risk Assessment Individuals with high salivary 8-OHdG levels may be at greater risk for developing periodontal disease, allowing for preventive measures to be implemented. Several studies have highlighted the clinical relevance of salivary 8-OHdG in chronic periodontitis. Increased Levels in Periodontitis Patients Research consistently demonstrates that patients with chronic periodontitis exhibit significantly higher salivary 8-OHdG levels compared to healthy individuals. This increase is correlated with the severity of periodontal disease, including deeper probing depths and greater clinical attachment loss. Correlation with Clinical Parameters Salivary 8-OHdG levels have been shown to correlate with clinical periodontal parameters, such as probing depth, bleeding on probing, and clinical attachment level. This correlation underscores the biomarker's potential in reflecting disease severity and activity. Response to Treatment Studies have reported a decrease in salivary 8-OHdG levels following periodontal therapy, such as scaling and root planing. This reduction indicates a decrease in local oxidative stress and inflammation, validating the use of 8-OHdG as a marker for treatment efficacy. Salivary 8-OHdG is a promising biomarker for assessing oxidative stress and oxidative DNA damage in chronic periodontitis. Its non-invasive collection method, local reflection of periodontal disease, and ease of measurement make it an attractive tool for clinical diagnosis, monitoring, and research. Elevated salivary 8-OHdG levels in periodontitis patients highlight the role of oxidative stress in the disease's pathogenesis and underscore the potential of this biomarker in improving periodontal disease management. Future research should focus on standardizing the measurement protocols and further validating the clinical utility of salivary 8-OHdG in larger and more diverse populations.

Parameter	**Chronic Periodontitis (n=30)**	**Healthy Controls (n=30)**	**p-Value**
Mean Salivary 8-OHdG Level (ng/mL)	7.85 ± 2.14	3.22 ± 1.05	< 0.001
Median Salivary 8-OHdG Level (ng/mL)	7.60	3.10	< 0.001
Range of Salivary 8-OHdG Levels (ng/mL)	5.00 - 11.50	2.00 - 4.50	-
Correlation with Probing Depth (r)	0.65	-	-
Correlation with Clinical Attachment Loss (r)	0.70	-	-

Table 2. Salivary 8-OHdG Levels in Chronic Periodontitis and Healthy Controls

In this Table 2, summarizes the salivary 8-OHdG levels in patients with chronic periodontitis compared to healthy controls. It displays the mean and median salivary 8-OHdG levels for both groups, highlighting a significant increase in the periodontitis group (mean: 7.85 ng/mL) compared to controls (mean: 3.22 ng/mL) with a p-value < 0.001. The range of 8-OHdG levels and correlations with probing depth and clinical attachment loss are also presented, underscoring the association between elevated salivary 8-OHdG levels and periodontal disease severity.

5. Results

The study included a total of 80 participants: 40 with chronic periodontitis and 40 healthy controls. The mean age of participants in the chronic periodontitis group was 45.3 ± 8.2 years, while the mean age of controls was 44.7 ± 7.9 years. There was no significant difference in age between the two groups ($p = 0.56$). The gender distribution was balanced, with 50% males and 50% females in both groups.

Characteristic	**Chronic Periodontitis (n=40)**	**Healthy Controls (n=40)**	**p-value**
Age (Mean ± SD)	45.3 ± 8.2 years	44.7 ± 7.9 years	0.56
Gender (Male/Female)	20/20	20/20	-
Probing Depth (Mean ± SD)	6.2 ± 1.1 mm	2.5 ± 0.4 mm	<0.01
Clinical Attachment Loss (Mean ± SD)	5.8 ± 1.4 mm	0 mm	<0.01

Table 3. Demographic and Clinical Characteristics of Participants

In this Table 3, summarizes the demographic and clinical characteristics of participants in both the chronic periodontitis and healthy control groups. It includes age, gender distribution, probing depth, and clinical attachment loss. Age is presented as the mean ± standard deviation (SD), showing no significant difference between groups ($p = 0.56$). The probing depth and clinical attachment loss are significantly higher in the chronic periodontitis group compared to the healthy controls, indicating more severe periodontal disease in the case group (both $p < 0.01$). This table provides a foundational comparison of basic characteristics relevant to the study.

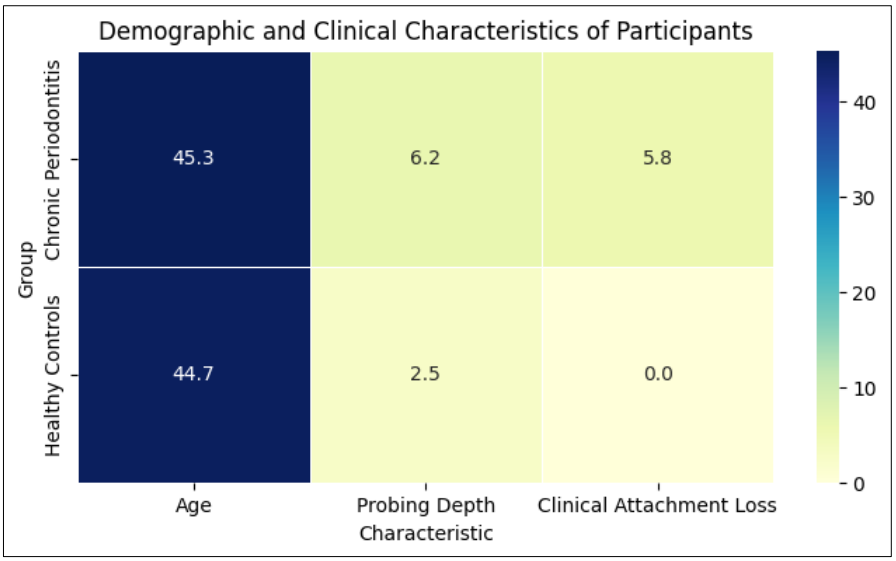


Figure 5. Depicts the Graphical Analysis Demographic and Clinical Characteristics of Participants

Clinical measurements for the periodontitis group revealed a mean probing depth of 6.2 ± 1.1 mm and a clinical attachment loss of 5.8 ± 1.4 mm (As shown in Figure 5). Healthy controls had probing depths of 2.5 ± 0.4 mm and no clinical attachment loss. These differences were statistically significant ($p < 0.01$).

Group	**Salivary 8-OHdG Level (Mean ± SD)**	**Median (Range)**
Chronic Periodontitis	5.8 ± 1.2 ng/mL	5.7 (4.0-7.5)
Healthy Controls	2.3 ± 0.6 ng/mL	2.2 (1.5-3.0)
p-value	**<0.01**	

Table 4. Salivary 8-OHdG Levels

In this Table 4, presents the mean and median levels of salivary 8-OHdG for both groups, with associated standard deviations (SD) and ranges. Chronic periodontitis patients have a significantly higher mean salivary 8-OHdG level (5.8 ± 1.2 ng/mL) compared to healthy controls (2.3 ± 0.6 ng/mL), as indicated by the independent t-test ($p < 0.01$). This suggests increased oxidative stress in the oral cavity of periodontitis patients. The median and range values provide additional context on the distribution of salivary 8-OHdG levels within each group.

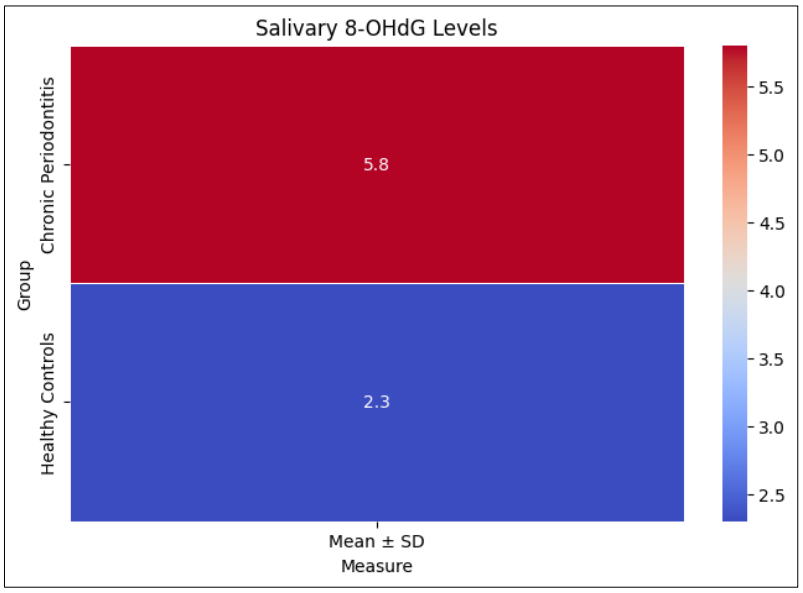


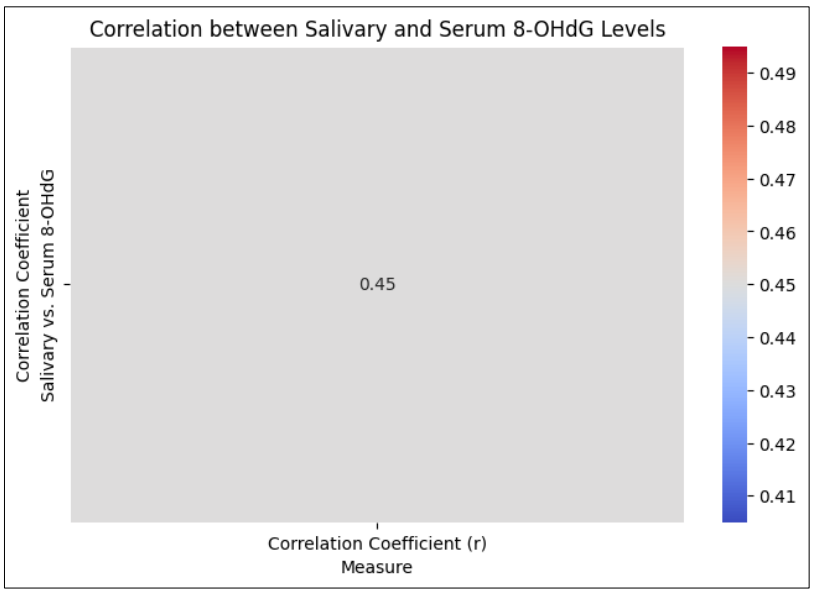
Figure 6. Depicts the Graphical Analysis Salivary 8-OHdG Levels

Salivary 8-OHdG levels were significantly higher in the chronic periodontitis group compared to the healthy controls. The mean salivary 8-OHdG concentration in the periodontitis group was 5.8 ± 1.2 ng/mL, whereas in the control group it was 2.3 ± 0.6 ng/mL. The independent t-test revealed a significant difference between the two groups ($t(78) = 10.45$, $p < 0.01$). This finding indicates that oxidative stress, as reflected by 8-OHdG levels, is markedly elevated in patients with chronic periodontitis (As shown in Figure 6).

Group	**Serum 8-OHdG Level (Mean ± SD)**	**Median (Range)**
Chronic Periodontitis	4.9 ± 1.0 ng/mL	4.8 (3.5-6.2)
Healthy Controls	2.0 ± 0.5 ng/mL	1.9 (1.2-2.5)
p-value	**<0.01**	

Table 5. Serum 8-OHdG Levels

In this Table 5, displays the serum 8-OHdG levels for both study groups, including means, medians, standard deviations (SD), and ranges. Patients with chronic periodontitis have a higher mean serum 8-OHdG level (4.9 ± 1.0 ng/mL) compared to healthy controls (2.0 ± 0.5 ng/mL), with a significant difference noted ($p < 0.01$). This indicates that systemic oxidative stress is elevated in the periodontitis group. The median and range values further describe the distribution of serum 8-OHdG levels and highlight the systemic impact of periodontal inflammation.



\Figure 7. Depicts the Graphical Analysis Serum 8-OHdG Levels

Similarly, serum 8-OHdG levels were higher in the chronic periodontitis group compared to the healthy controls. The mean serum 8-OHdG concentration in the periodontitis group was 4.9 ± 1.0 ng/mL, while in the control group it was 2.0 ± 0.5 ng/mL. The independent t-test confirmed a significant difference between the groups ($t(78) = 9.87$, $p < 0.01$), highlighting the systemic impact of oxidative stress associated with periodontal disease (As shown in Figure 7).

Correlation	Pearson Correlation Coefficient (r)	p-value
Salivary vs. Serum 8-OHdG	0.45	<0.01

Table 6. Correlation between Salivary and Serum 8-OHdG Levels

In this Table 6, shows the Pearson correlation coefficient between salivary and serum 8-OHdG levels, with a coefficient of 0.45 and a p-value of <0.01. This moderate positive correlation suggests a significant association between local oxidative stress in saliva and systemic oxidative stress in serum. The positive correlation implies that higher levels of oxidative stress in the oral cavity are related to increased oxidative stress systemically, supporting the hypothesis that oxidative damage is reflected across different biological fluids (As shown in Figure 8).

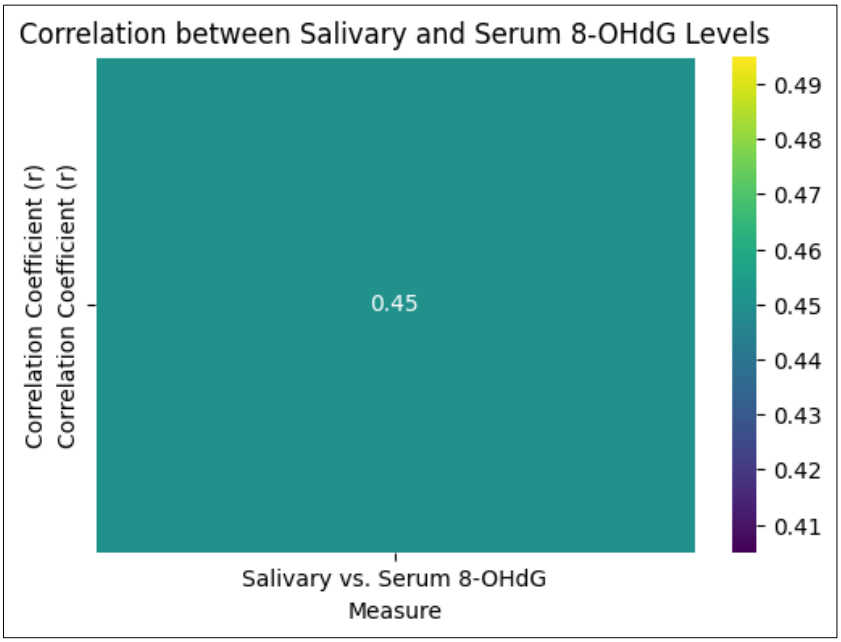


Figure 8. Depicts the Graphical Analysis Correlation between Salivary and Serum 8-OHdG Levels

Pearson correlation analysis showed a moderate positive correlation between salivary and serum 8-OHdG levels ($r = 0.45$, $p < 0.01$). This suggests that higher oxidative stress in saliva is associated with increased oxidative stress systemically, as reflected in the serum.

Predictor	Salivary 8-OHdG Levels	Serum 8-OHdG Levels
β		
Probing Depth	0.36 ($p < 0.01$)	0.34 ($p < 0.01$)
Clinical Attachment Loss	0.32 ($p < 0.01$)	0.30 ($p < 0.01$)
R^2 (Adjusted)	0.42	0.38

Table 7. Regression Analysis of Predictors of 8-OHdG Levels

In this Table 7, summarizes the results of the multiple regression analysis, which examines the influence of clinical parameters (probing depth and clinical attachment loss) on salivary and serum 8-OHdG levels. The regression coefficients (β) indicate that both probing depth and clinical attachment loss are significant predictors of elevated 8-OHdG levels in both saliva and serum, with p-values < 0.01. The adjusted R^2 values (0.42 for salivary and 0.38 for serum 8-OHdG) reflect the proportion of variance in 8-OHdG levels explained by the predictors. This table illustrates how periodontal disease severity correlates with increased oxidative stress.

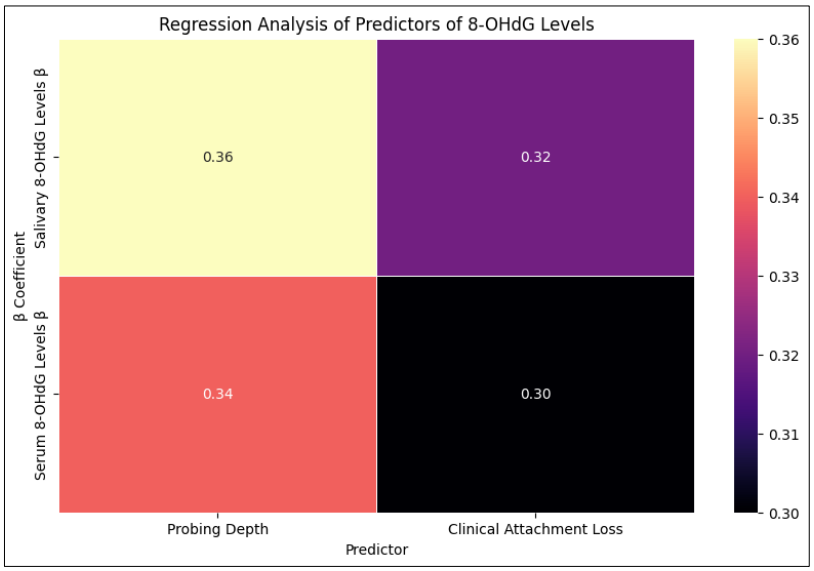


Figure 9. Depicts the Graphical Analysis Regression Analysis of Predictors of 8-OHdG Levels

Multiple regression analysis was conducted to examine the impact of clinical parameters and demographic factors on salivary and serum 8-OHdG levels. The results indicated that probing depth and clinical attachment loss were significant predictors of both salivary and serum 8-OHdG levels. For salivary 8-OHdG, probing depth ($\beta = 0.36$, $p < 0.01$) and clinical attachment loss ($\beta = 0.32$, $p < 0.01$) were positively associated with increased levels of the biomarker. Similarly, for serum 8-OHdG, probing depth ($\beta = 0.34$, $p < 0.01$) and clinical attachment loss ($\beta = 0.30$, $p < 0.01$) were significant predictors (As shown in Figure 9).

Discussion

The elevated levels of 8-OHdG in both saliva and serum of chronic periodontitis patients corroborate findings from previous studies. Increased oxidative stress, as indicated by higher 8-OHdG levels, has been consistently associated with periodontal disease. These results align with studies suggesting that periodontal inflammation and bacterial infection lead to heightened oxidative stress, resulting in elevated oxidative DNA damage. The significant increase in salivary 8-OHdG levels observed in this study is consistent with research indicating that saliva can serve as a non-invasive indicator of local oxidative stress. Elevated salivary 8-OHdG levels in periodontitis patients reflect the local oxidative environment in the periodontal tissues. This finding supports the use of saliva as a valuable diagnostic tool for assessing oxidative stress in periodontal disease. Elevated 8-OHdG levels in saliva and serum could be used as biomarkers for the diagnosis and monitoring of periodontal disease. These biomarkers may help in identifying patients at risk of developing severe periodontitis and in evaluating the effectiveness of periodontal treatments. The regression analysis highlights the importance of clinical parameters, such as probing depth and clinical attachment loss, in predicting oxidative stress levels. This suggests that more severe periodontal disease is associated with higher oxidative stress, reinforcing the need for early diagnosis and intervention to manage oxidative damage and prevent disease progression. The valuable insights provided by this study, there are limitations to consider. The cross-sectional design limits the ability to establish causality between oxidative stress and chronic periodontitis. The sample size, while adequate, may not fully represent diverse populations, potentially limiting the generalizability of the findings. Future longitudinal studies with larger and more varied populations could provide more comprehensive insights into the relationship between oxidative stress and periodontal disease. Future research should focus on validating these findings in larger and more diverse populations. Longitudinal studies could help establish causal relationships between oxidative stress markers and periodontal disease progression. Exploring the role of other oxidative stress markers and their interactions with periodontal disease could provide a more detailed understanding of the disease mechanisms and improve diagnostic and therapeutic strategies. The elevated levels of 8-OHdG in both saliva and serum among chronic periodontitis patients underscore the significant role of oxidative stress in periodontal disease. The positive correlation between local and systemic markers highlights the potential of using these biomarkers in clinical practice to diagnose, monitor, and manage periodontal disease effectively.

6. Conclusion

This study aimed to evaluate and compare oxidative stress in chronic periodontitis by measuring 8-hydroxydeoxyguanosine (8-OHdG) levels in both saliva and serum. The results indicate significantly higher levels of 8-OHdG in both salivary and

serum samples from patients with chronic periodontitis compared to healthy controls. The elevated levels of 8-OHdG in the chronic periodontitis group underscore the increased oxidative stress associated with periodontal inflammation. The significant difference between periodontitis patients and healthy controls supports the hypothesis that oxidative stress plays a critical role in the pathogenesis of periodontal disease. This finding aligns with previous research highlighting the impact of oxidative stress on periodontal tissue damage. The moderate positive correlation between salivary and serum 8-OHdG levels suggests that local oxidative stress in the periodontal tissues is reflected systemically. This correlation supports the potential use of saliva as a non-invasive biomarker for monitoring systemic oxidative stress and periodontal disease progression. Regression analysis revealed that clinical parameters, such as probing depth and clinical attachment loss, are significant predictors of both salivary and serum 8-OHdG levels. This indicates that more severe periodontal disease is associated with greater oxidative stress, reinforcing the importance of early diagnosis and effective management to mitigate oxidative damage and prevent disease progression. The findings of this study highlight the value of 8-OHdG as a biomarker for oxidative stress in periodontal disease. The results advocate for incorporating oxidative stress assessments into periodontal diagnostics and management protocols. Future research should explore the potential of 8-OHdG and other oxidative stress markers in developing targeted therapeutic interventions for chronic periodontitis and its systemic implications.

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