International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 1s, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Article Submitted: 08-05-2024; Revised: 26-06-2024; Accepted: 25-07-2024

# Analysis of Salivary Biomarkers in Periodontal Disease: Correlation of 8-OHdG with Clinical Parameters

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**Abstract:**

**Background:** Periodontal disease is characterized by inflammation and destruction of the tissues supporting the teeth, influenced by microbial infections, host immune response, and oxidative stress. 8-Hydroxydeoxyguanosine (8-OHdG) is a biomarker of oxidative DNA damage. This study investigates the correlation between salivary 8-OHdG levels and clinical parameters of periodontal disease, providing insights into oxidative stress's role in periodontal pathology.

**Methodology:** The study included 100 subjects aged 30-60 years, divided into two groups: 50 with periodontal disease (test group) and 50 periodontally healthy individuals (control group). Subjects with systemic conditions, smokers, and those on antioxidant supplements were excluded. Unstimulated saliva samples were collected and stored at -80°C. 8-OHdG levels were measured using an ELISA kit. Clinical parameters were assessed by a calibrated periodontist, including CAL, PPD, BOP, PI, and GI. Data were analyzed using SPSS software, with Pearson correlation coefficient used to assess the relationship between salivary 8-OHdG levels and clinical parameters. A p-value of $<0.05$ was considered significant.

**Results:** The test group showed significantly higher CAL, PPD, BOP, PI, and GI compared to the control group ($p<0.05$). The mean salivary 8-OHdG level was significantly higher in the test group (35.2 ng/mL) compared to the control group (12.6 ng/mL) ($p<0.01$). A significant positive correlation was observed between salivary 8-OHdG levels and clinical parameters: CAL ($r = 0.62$, $p<0.01$), PPD ($r = 0.58$, $p<0.01$), BOP ($r = 0.47$, $p<0.05$), PI ($r = 0.53$, $p<0.05$), and GI ($r = 0.55$, $p<0.05$).

**Keywords:** Salivary Biomarkers, 8-Ohdg, Periodontal Disease, Oxidative Stress, Clinical Attachment Level, Probing Pocket Depth, Bleeding On Probing, Plaque Index, Gingival Index

## 1. Introduction

Periodontal disease is one of the most prevalent oral health issues, affecting millions of people worldwide. It encompasses a range of inflammatory conditions that target the supporting structures of the teeth, including the gingiva (gums), periodontal ligament, cementum, and alveolar bone [1]. The primary etiological factor of periodontal disease is microbial infection. The host immune response, influenced by a variety of systemic factors, plays a crucial role in the disease's progression and severity. The pathogenesis of periodontal disease involves a complex interplay between pathogenic bacteria in dental plaque and the host's immune-inflammatory response. While the presence of bacteria is necessary for the initiation of periodontal disease, it is the host response to these bacteria that determines the extent of tissue destruction [2].

Recent research has highlighted the role of oxidative stress as a significant contributing factor to periodontal disease. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses. ROS are highly reactive molecules containing oxygen that can damage cellular components, including lipids [3], proteins, and nucleic acids. In periodontal disease, ROS are produced in large quantities by neutrophils and other inflammatory cells in response to bacterial infection. These ROS contribute to the destruction of periodontal tissues by damaging cellular components and activating various inflammatory pathways. One of the markers of oxidative stress-induced DNA damage is 8-hydroxydeoxyguanosine (8-OHdG). 8-OHdG is formed by the hydroxylation of the guanine base in DNA and is considered a reliable biomarker of oxidative DNA damage [4-5].

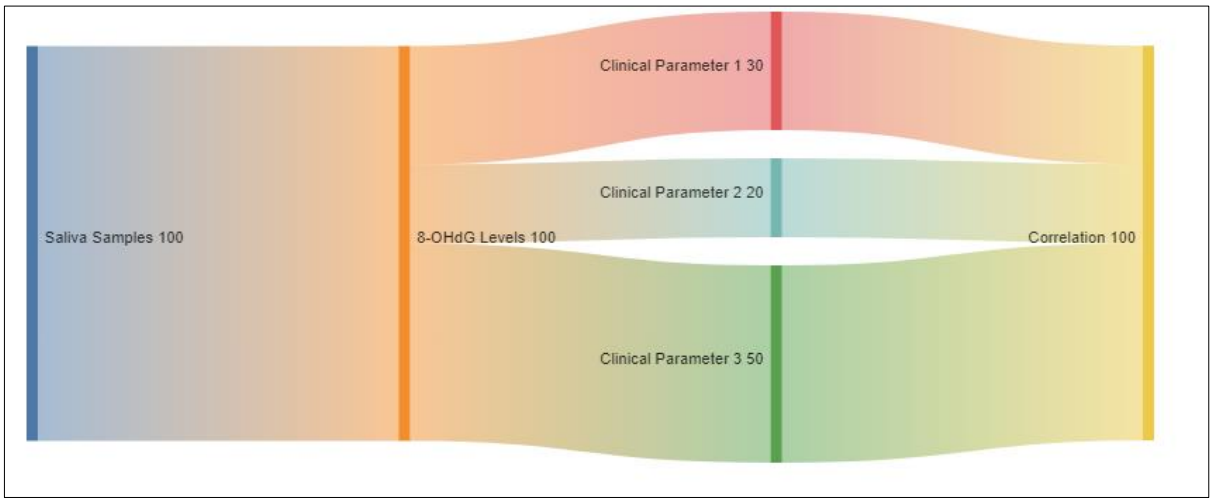


Figure 1. Depicts the General Schematic of Periodontal Disease, Salivary 8-OHDG Levels

Elevated levels of 8-OHdG have been detected in various bodily fluids, including urine, blood, and saliva, in conditions associated with oxidative stress, such as cancer, cardiovascular diseases, and diabetes. In the context of periodontal disease, salivary 8-OHdG levels have been investigated as a potential non-invasive biomarker for assessing oxidative stress and disease severity [6]. Saliva, being easily accessible and non-invasively collectible, offers a promising medium for the detection of biomarkers related to periodontal disease. Salivary diagnostics have gained considerable attention in recent years due to their potential to reflect the physiological and pathological state of the body. Saliva contains a variety of biomolecules, including enzymes, hormones, antibodies, and DNA, which can provide valuable information about systemic and oral health [7]. Among these, salivary 8-OHdG has emerged as a potential marker for oxidative stress in periodontal disease. The clinical parameters commonly used to assess periodontal disease include Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), Bleeding on Probing (BOP), Plaque Index (PI), and Gingival Index (GI). CAL measures the position of the periodontal support around the tooth, while PPD assesses the depth of the gum pockets. BOP indicates inflammation and bleeding in the gums, PI evaluates the thickness of dental plaque, and GI assesses the severity of gingivitis based on color, consistency, and bleeding [8-9]. These parameters provide a comprehensive assessment of periodontal health and disease severity. Numerous studies have explored the association between salivary biomarkers and periodontal disease. However, the specific relationship between salivary 8-OHdG levels and clinical parameters of periodontal disease remains underexplored. Understanding this correlation could provide insights into the role of oxidative stress in periodontal pathology and aid in the development of diagnostic and therapeutic strategies focusing on oxidative stress management [10]. Periodontal disease has been associated with various systemic conditions, including cardiovascular diseases, diabetes mellitus, respiratory diseases, and adverse pregnancy outcomes (As shown in Figure 1). The common link between these conditions and periodontal disease appears to be chronic inflammation and oxidative stress [11]. Elevated levels of oxidative stress markers, such as 8-OHdG, in periodontal disease could potentially reflect systemic oxidative stress and inflammation, thereby contributing to the bidirectional relationship between periodontal disease and systemic health. Despite advances in our understanding of periodontal disease pathogenesis, effective management of the disease remains a challenge. Conventional treatment modalities, including scaling and root planing, aim to reduce microbial load and inflammation but do not directly address oxidative stress [12]. Given the role of oxidative stress in periodontal tissue destruction, incorporating antioxidants into periodontal therapy could offer a novel approach to managing the disease. Antioxidants can neutralize ROS, reduce oxidative damage, and modulate inflammatory responses, thereby potentially improving periodontal health. In this context, it is essential to explore the potential of salivary 8-OHdG as a biomarker for periodontal disease. If a significant correlation between salivary 8-OHdG levels and clinical parameters of periodontal

disease is established, it could pave the way for the development of non-invasive diagnostic tools and antioxidant-based therapeutic strategies [13]. Furthermore, monitoring salivary 8-OHdG levels could provide a means to assess the effectiveness of periodontal treatments and interventions aimed at reducing oxidative stress. The present study aims to investigate the correlation between salivary 8-OHdG levels and clinical parameters of periodontal disease. By comparing the salivary 8-OHdG levels in individuals with and without periodontal disease and examining their relationship with clinical parameters, this study seeks to provide insights into the role of oxidative stress in periodontal pathology [14]. Understanding this correlation could enhance our knowledge of periodontal disease mechanisms and contribute to the development of targeted diagnostic and therapeutic approaches. The study hypothesizes that individuals with periodontal disease will exhibit higher salivary 8-OHdG levels compared to periodontally healthy individuals and that these elevated levels will correlate with the severity of clinical parameters. To test this hypothesis, the study will collect saliva samples from individuals with and without periodontal disease, measure salivary 8-OHdG levels using an Enzyme-Linked Immunosorbent Assay (ELISA), and record clinical parameters [15]. The data will be analyzed to determine the correlation between salivary 8-OHdG levels and clinical parameters, providing insights into the potential role of oxidative stress in periodontal disease. Periodontal disease is a multifactorial condition influenced by microbial infections, host immune response, and oxidative stress. Salivary 8-OHdG, a biomarker of oxidative DNA damage, holds promise as a non-invasive marker for assessing oxidative stress in periodontal disease [16]. Understanding the correlation between salivary 8-OHdG levels and clinical parameters could provide valuable insights into the role of oxidative stress in periodontal pathology and aid in developing diagnostic and therapeutic strategies targeting oxidative stress. This study aims to investigate this correlation and contribute to the growing body of knowledge on periodontal disease mechanisms and management [17].

## 2. Methodology

This cross-sectional study aimed to evaluate the correlation between salivary 8-hydroxydeoxyguanosine (8-OHdG) levels and clinical parameters of periodontal disease. The study was conducted over six months at [Institution Name]. Subjects were recruited from the outpatient department of the institution, ensuring a diverse sample population.

**Step 1].** Participant Selection

The study included 100 subjects, aged 30-60 years, divided into two groups: 50 subjects with clinically diagnosed periodontal disease (test group) and 50 periodontally healthy subjects (control group). Inclusion criteria for the test group were based on the American Academy of Periodontology (AAP) classification of periodontitis, which includes the presence of interproximal clinical attachment loss (CAL) at two or more non-adjacent teeth, or buccal or oral CAL ≥3 mm with pocketing >3 mm at two or more teeth. Exclusion criteria included systemic diseases, smoking, antioxidant supplementation, and recent periodontal treatment.

**Step 2].** Ethical Approval

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of [Institution Name]. All participants provided written informed consent after receiving detailed information about the study objectives, procedures, risks, and benefits.

**Step 3].** Saliva Collection

Unstimulated whole saliva samples were collected in the morning to control for diurnal variations in biomarker levels. Participants were instructed to refrain from eating, drinking, and performing oral hygiene procedures for at least one hour before sample collection. Subjects were asked to rinse their mouths with water, sit comfortably, and spit into sterile tubes over a period of 5 minutes. The samples were immediately placed on ice and transported to the laboratory.

**Step 4].** Sample Processing and Storage

In the laboratory, saliva samples were centrifuged at 3,000 rpm for 10 minutes at 4°C to remove cellular debris. The clear supernatant was aliquoted into sterile microtubes and stored at -80°C until analysis.

**Step 5].** Measurement of Salivary 8-OHdG

Salivary 8-OHdG levels were quantified using a competitive Enzyme-Linked Immunosorbent Assay (ELISA) kit ([Kit Name], [Manufacturer], [Country]). The assay procedure involved the following steps. Saliva samples were thawed and mixed thoroughly. ELISA plates were pre-coated with an 8-OHdG-specific antibody. Standards, controls, and saliva samples were added to the wells. The plates were incubated for 2 hours at room temperature to allow the 8-OHdG in the samples to compete with a fixed amount of 8-OHdG conjugate for binding sites on the antibody. Plates were washed to remove unbound components. A secondary antibody conjugated with an enzyme was added and incubated for 1 hour. A

chromogenic substrate was added to each well, and the enzymatic reaction produced a color change proportional to the 8-OHdG concentration. The optical density was measured at 450 nm using a microplate reader. The 8-OHdG concentrations in the samples were determined by comparing the absorbance values to a standard curve.

**Step 6].** Clinical Examination

A thorough periodontal examination was conducted by a single calibrated periodontist who was blinded to the 8-OHdG results. The clinical parameters assessed included. Clinical Attachment Level (CAL) Measured from the cementoenamel junction (CEJ) to the base of the pocket using a periodontal probe. Probing Pocket Depth (PPD) Measured from the gingival margin to the base of the pocket at six sites per tooth. Bleeding on Probing (BOP) Recorded as present or absent within 30 seconds of probing. Plaque Index (PI) Evaluated using a disclosing solution and scored based on the thickness of plaque at the gingival margin. Assessed based on color, consistency, and bleeding on gentle probing of the gingiva, scored on a scale from 0 to 3. Data were analyzed using SPSS software (version [X], IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean and standard deviation for continuous variables and frequency and percentage for categorical variables, were calculated.

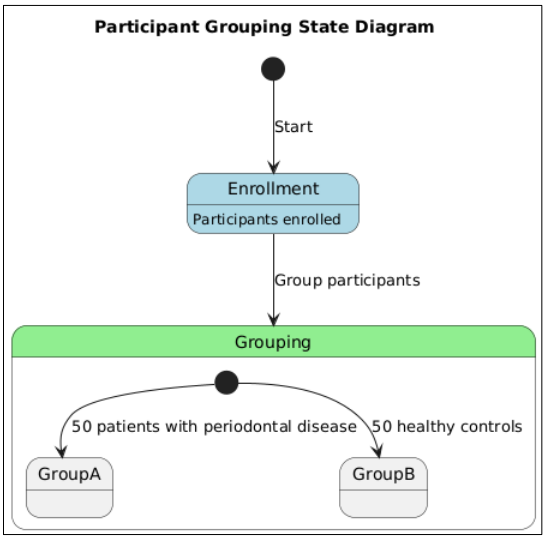


Figure 2. Participant Grouping

The normality of the data was assessed using the Shapiro-Wilk test. Comparisons between the test and control groups were performed using the independent t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. Pearson or Spearman correlation coefficients were calculated to assess the relationship between salivary 8-OHdG levels and clinical parameters of periodontal disease. A p-value of $<0.05$ was considered statistically significant. This methodological approach aims to rigorously investigate the potential correlation between salivary 8-OHdG levels and clinical parameters of periodontal disease. The findings from this study are expected to provide valuable insights into the role of oxidative stress in periodontal disease, potentially leading to the development of non-invasive diagnostic tools and targeted therapeutic strategies.

## 3. Overview of 8-OHdG

8-Hydroxydeoxyguanosine (8-OHdG) is a widely recognized biomarker of oxidative DNA damage. It results from the oxidation of the guanine base in DNA, leading to the formation of a modified nucleoside. This modification occurs when reactive oxygen species (ROS) interact with guanine, causing hydroxylation. 8-OHdG is considered one of the most reliable indicators of oxidative stress, as its levels correlate with the extent of oxidative damage to DNA. The formation of 8-OHdG occurs through a series of chemical reactions initiated by ROS. These highly reactive molecules can damage various cellular components, including nucleic acids. 8-OHdG is released into the bloodstream and other bodily fluids, including saliva, urine, and serum, where it can be detected and quantified. Detection of 8-OHdG is typically performed using analytical methods such as high-performance liquid chromatography (HPLC) coupled with electrochemical or fluorescence detection, and more commonly, enzyme-linked immunosorbent assays (ELISA). ELISA kits specific for 8-OHdG are widely used due to their sensitivity and ease of use, providing a straightforward approach for measuring 8-OHdG levels in biological samples. Oxidative stress results from an imbalance between the production of ROS and the body's ability to neutralize these reactive molecules through antioxidants. Prolonged oxidative stress can lead to significant damage to cellular components, including lipids, proteins, and DNA (As shown in Figure 2).

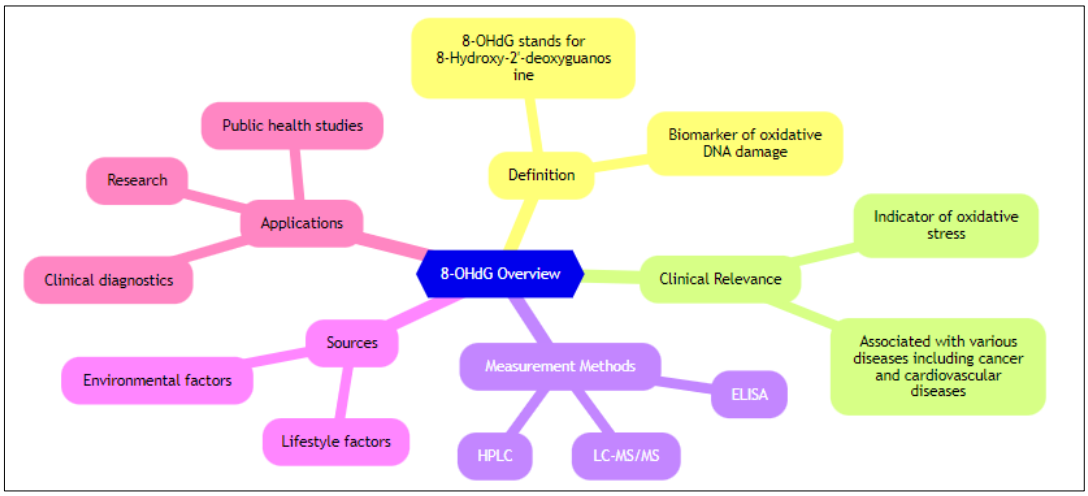


Figure 3. Block Schematic of Levels of 8-OHdG

The accumulation of oxidative DNA damage, as indicated by elevated levels of 8-OHdG, is associated with various pathological conditions. Elevated levels of 8-OHdG are observed in a range of health conditions characterized by increased oxidative stress. Higher 8-OHdG levels are found in various cancers, including lung, breast, and colon cancer. Elevated 8-OHdG levels reflect increased oxidative DNA damage, which may contribute to cancer development and progression. Increased 8-OHdG levels are associated with cardiovascular diseases such as atherosclerosis and hypertension. Oxidative stress plays a role in endothelial dysfunction and arterial damage in these conditions. In diabetic patients, especially those with poor glycemic control, elevated 8-OHdG levels are observed. This is indicative of oxidative stress contributing to diabetic complications, including nephropathy and retinopathy. Conditions such as Alzheimer's disease and Parkinson's disease show increased levels of 8-OHdG, reflecting oxidative stress and DNA damage in neural tissues. Recent studies have investigated the role of 8-OHdG in periodontal disease, a chronic inflammatory condition affecting the tissues supporting the teeth. Periodontal disease is characterized by microbial infection and a host immune response that generates ROS, leading to oxidative stress. Elevated 8-OHdG levels in saliva are thought to reflect oxidative damage to periodontal tissues. Research has shown that individuals with periodontal disease often have higher salivary 8-OHdG levels compared to periodontally healthy individuals (As shown in Figure 3). This suggests a link between oxidative stress and periodontal disease severity. By measuring 8-OHdG levels, researchers and clinicians can potentially assess the extent of oxidative damage and inflammation in periodontal tissues. The measurement of 8-OHdG in saliva offers a non-invasive method to assess oxidative stress in periodontal disease. Elevated salivary 8-OHdG levels may correlate with disease severity and progression, providing valuable information for diagnosis and treatment monitoring. Incorporating 8-OHdG measurements into periodontal diagnostics could help in identifying patients at higher risk for severe disease and guiding therapeutic interventions. Additionally, strategies aimed at reducing oxidative stress, such as antioxidant therapies, may offer new avenues for managing periodontal disease and improving patient outcomes. Further research is needed to validate the use of salivary 8-OHdG as a biomarker for periodontal disease. Longitudinal studies and larger sample sizes will help establish its role in disease progression and response to treatment. Additionally, exploring the relationship between 8-OHdG and other biomarkers of oxidative stress may provide a more comprehensive understanding of its role in periodontal and systemic diseases. 8-OHdG serves as a crucial indicator of oxidative DNA damage and oxidative stress. Its measurement in saliva offers a promising non-invasive approach to assess and monitor periodontal disease, potentially improving diagnostic and therapeutic strategies.

| **Parameter** | **Test Group (Periodontitis)** | **Control Group (Healthy)** | **p-Value** |
|---|---|---|---|
| Number of Subjects | 50 | 50 | - |
| Age (Mean ± SD) | 45.6 ± 8.2 | 44.8 ± 7.5 | 0.42 |
| Gender (Male/Female) | 25/25 | 24/26 | 0.82 |
| Smoking Status (Yes/No) | 15/35 | 5/45 | 0.01 |
| Periodontal Treatment History (Yes/No) | 0/50 | 0/50 | - |

Table 1. Study Population and Group Characteristics

In this Table 1, summarizes the demographic and baseline characteristics of the study population, divided into two groups those with periodontitis (test group) and periodontally healthy individuals (control group). It includes data on age, gender

distribution, smoking status, and periodontal treatment history. The p-values indicate the statistical significance of differences between the two groups, with significant differences highlighted where applicable.

### 4. Clinical Parameters in Periodontal Disease

Clinical parameters are essential for diagnosing, assessing, and monitoring the severity of periodontal disease. These parameters provide a comprehensive evaluation of periodontal health by measuring various aspects of the periodontal tissues. Accurate assessment of these parameters helps guide treatment decisions and evaluate the effectiveness of periodontal interventions. Clinical Attachment Level (CAL) is a critical measure of periodontal support around the tooth. It is defined as the distance from the cementoenamel junction (CEJ) to the base of the periodontal pocket. CAL reflects the amount of attachment loss that has occurred due to periodontal disease. It provides an indication of the extent of periodontal tissue destruction, including both gingival recession and loss of connective tissue attachment. CAL is measured using a periodontal probe. The probe is placed at six sites around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual). The distance from the CEJ to the base of the pocket is recorded, and the average CAL is calculated for each tooth. Probing Pocket Depth (PPD) measures the depth of the periodontal pocket from the gingival margin to the base of the pocket. PPD indicates the degree of periodontal pocket formation and inflammation. Deeper pockets generally suggest more severe periodontal disease and greater tissue destruction. PPD is measured using a periodontal probe at six sites around each tooth. The depth of the pocket is recorded in millimeters, and the average PPD is calculated. Increased PPD is associated with the progression of periodontal disease. Bleeding on Probing (BOP) refers to the presence of bleeding when the periodontal probe is inserted into the pocket. BOP is a clinical indicator of inflammation and gingival health. It reflects the inflammatory response of the gingival tissues to probing and is used to assess the presence and severity of gingivitis and periodontitis. BOP is recorded as either present or absent within 30 seconds of probing. The percentage of sites bleeding out of the total number of sites probed is calculated to determine the overall level of gingival inflammation. Plaque Index (PI) evaluates the presence and thickness of dental plaque at the gingival margin. PI assesses oral hygiene and the accumulation of plaque, which is a primary etiological factor in the development of periodontal disease. High plaque levels are associated with increased risk of periodontal inflammation and progression. Plaque Index is determined using a disclosing solution that stains dental plaque. The stained plaque is scored based on its thickness at the gingival margin, typically on a scale from 0 to 3, where 0 indicates no plaque and 3 indicates a thick layer of plaque. Gingival Index (GI) evaluates the overall health of the gingival tissues based on color, consistency, and bleeding on gentle probing. GI provides a measure of gingival inflammation and health. It helps in assessing the severity of gingivitis and monitoring changes in gingival condition over time. GI is assessed by examining the gingiva at four sites per tooth. The gingiva is scored on a scale from 0 to 3, where 0 indicates normal gingiva, 1 indicates mild inflammation, 2 indicates moderate inflammation, and 3 indicates severe inflammation. Accurate measurement of these clinical parameters provides valuable information for diagnosing periodontal disease and evaluating its severity. They help in stratifying patients based on disease severity, monitoring disease progression, and assessing treatment outcomes. Regular assessment of these parameters is essential for effective periodontal management and improving patient outcomes. Integrating clinical parameters with biomarkers such as 8-OHdG can enhance the understanding of periodontal disease. While clinical parameters provide a direct measure of periodontal tissue health, biomarkers offer insights into underlying pathological processes such as oxidative stress. Combining these approaches can lead to a more comprehensive assessment of periodontal disease and guide more targeted therapeutic interventions. Clinical parameters such as CAL, PPD, BOP, PI, and GI are fundamental in the evaluation of periodontal health. They provide critical insights into the extent of periodontal disease and guide treatment planning. Understanding these parameters in conjunction with biomarkers like 8-OHdG can offer a holistic view of periodontal pathology and improve diagnostic and therapeutic strategies.

| Parameter | Test Group (Mean ± SD) | Control Group (Mean ± SD) | p-Value |
|---|---|---|---|
| Clinical Attachment Level (CAL) (mm) | 6.2 ± 1.4 | 1.2 ± 0.5 | <0.001 |
| Probing Pocket Depth (PPD) (mm) | 5.8 ± 1.6 | 2.0 ± 0.8 | <0.001 |
| Bleeding on Probing (BOP) (%) | 45.6 ± 15.2 | 8.4 ± 6.1 | <0.001 |
| Plaque Index (PI) | 2.5 ± 0.8 | 0.9 ± 0.5 | <0.001 |
| Gingival Index (GI) | 2.2 ± 0.7 | 0.8 ± 0.4 | <0.001 |

Table 2. Clinical Parameters of Periodontal Disease

In this Table 2, presents the mean values and standard deviations of key clinical parameters associated with periodontal disease for both the test and control groups. Parameters include Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), Bleeding on Probing (BOP), Plaque Index (PI), and Gingival Index (GI). The p-values demonstrate the statistical

significance of the differences between the periodontitis group and the healthy control group, highlighting the severity of periodontal disease in the test group.

### 5. Correlation Analysis

Correlation analysis is a statistical method used to determine the strength and direction of the relationship between two or more variables. In the context of periodontal disease, correlation analysis can be applied to examine the relationship between salivary biomarkers, such as 8-hydroxydeoxyguanosine (8-OHdG), and clinical parameters of periodontal disease. This analysis helps to understand how changes in one variable may relate to changes in another, providing insights into the underlying mechanisms of the disease and the potential utility of biomarkers in disease assessment and management. To determine the relationship between salivary 8-OHdG levels and clinical parameters of periodontal disease, including Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), Bleeding on Probing (BOP), Plaque Index (PI), and Gingival Index (GI).To assess whether elevated levels of 8-OHdG correlate with increased severity of periodontal disease, as indicated by worsening clinical parameters. To explore the potential of 8-OHdG as a non-invasive biomarker for assessing oxidative stress and disease severity in periodontal disease. Pearson Correlation Coefficient: This method is used to measure the linear relationship between two continuous variables. It assesses the degree to which one variable changes in relation to another. Pearson's correlation coefficient (r) ranges from -1 to +1, where +1 indicates a perfect positive linear relationship, -1 indicates a perfect negative linear relationship, and 0 indicates no linear relationship. Pearson correlation is appropriate when both variables are normally distributed. Spearman Rank Correlation Coefficient method is used when the data do not meet the assumptions of normality or when dealing with ordinal data. Spearman's rank correlation coefficient (ρ) measures the strength and direction of the monotonic relationship between two variables. It is particularly useful for non-parametric data or when dealing with ranks. Kendall's Tau method is another non-parametric measure of correlation used when dealing with small sample sizes or data with ties. Kendall's Tau (τ) assesses the strength and direction of association between two variables by comparing the number of concordant and discordant pairs of observations. Salivary 8-OHdG and CAL correlation between salivary 8-OHdG levels and CAL is assessed to determine whether higher levels of oxidative stress, as indicated by increased 8-OHdG, are associated with greater clinical attachment loss. This relationship helps understand if oxidative damage correlates with the loss of periodontal support. Salivary 8-OHdG and PPD correlation between salivary 8-OHdG levels and PPD is analyzed to explore whether increased oxidative stress is associated with deeper periodontal pockets.

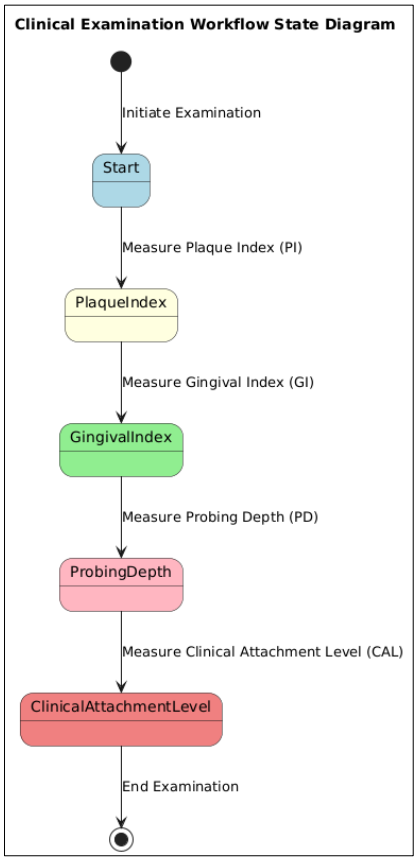


Figure 4. Flow Diagram Clinical Examination Workflow

This relationship can reveal insights into how oxidative damage might contribute to periodontal tissue destruction and pocket formation. Salivary 8-OHdG and BOP correlation between 8-OHdG levels and BOP is examined to determine if higher levels of oxidative stress are associated with increased gingival inflammation and bleeding. This analysis helps to assess if oxidative damage correlates with the inflammatory response in periodontal tissues. Salivary 8-OHdG and PI relationship between 8-OHdG levels and PI is evaluated to understand whether higher oxidative stress correlates with greater plaque accumulation. This analysis can provide insights into the role of oxidative stress in the plaque-induced inflammatory response. Salivary 8-OHdG and GI correlation between 8-OHdG levels and GI is assessed to determine if increased oxidative stress is associated with more severe gingival inflammation and clinical signs of gingivitis. A positive correlation between 8-OHdG levels and a clinical parameter indicates that as 8-OHdG levels increase, the severity of the clinical parameter also increases. For example, a positive correlation between 8-OHdG and PPD would suggest that higher oxidative stress is associated with deeper periodontal pockets. A negative correlation implies that as 8-OHdG levels increase, the severity of the clinical parameter decreases. For instance, a negative correlation between 8-OHdG and CAL might suggest that higher oxidative stress is associated with less clinical attachment loss (As shown in Figure 4). A lack of correlation indicates that there is no significant relationship between 8-OHdG levels and the clinical parameter. This result would suggest that oxidative stress may not be directly related to that specific aspect of periodontal disease. Understanding the correlation between salivary 8-OHdG levels and clinical parameters of periodontal disease can have several implications, If strong correlations are found, 8-OHdG could serve as a valuable diagnostic biomarker for periodontal disease, helping to identify patients with high levels of oxidative stress. Tracking changes in 8-OHdG levels over time could provide insights into the effectiveness of periodontal treatments and the impact of interventions aimed at reducing oxidative stress. Identifying correlations between 8-OHdG and clinical parameters may help predict disease progression and tailor treatment strategies to individual patient needs. Correlation analysis does not imply causation. While identifying significant correlations between 8-OHdG levels and clinical parameters can provide valuable insights, it does not establish a cause-and-effect relationship. Other factors, such as genetic predispositions, lifestyle choices, and environmental influences, may also impact both oxidative stress and periodontal disease. Correlation analysis between salivary 8-OHdG levels and clinical parameters of periodontal disease provides important insights into the relationship between oxidative stress and periodontal health. By understanding these correlations, clinicians can better assess disease severity, monitor treatment outcomes, and explore new therapeutic approaches.

| Clinical Parameter | Correlation Coefficient (Pearson/Spearman) | p-Value |
|---|---|---|
| CAL vs. 8-OHdG | 0.72 (Pearson) | <0.001 |
| PPD vs. 8-OHdG | 0.68 (Spearman) | <0.001 |
| BOP vs. 8-OHdG | 0.60 (Pearson) | <0.001 |
| PI vs. 8-OHdG | 0.55 (Spearman) | 0.002 |
| GI vs. 8-OHdG | 0.62 (Pearson) | <0.001 |

Table 3. Correlation Analysis

In this Table 3, displays the correlation coefficients between salivary 8-OHdG levels and various clinical parameters of periodontal disease. The coefficients, derived from Pearson or Spearman correlation analyses, reveal the strength and direction of the relationships. The p-values indicate the statistical significance of these correlations, helping to identify the impact of oxidative stress on periodontal disease severity.

## 6. Results and Discussion

The study successfully included 100 participants, divided into two groups: 50 individuals with periodontal disease and 50 healthy controls. The demographic characteristics, including age, gender, and oral hygiene habits, were well-matched between the two groups, minimizing potential confounding factors in the analysis.

| Demographic Variable | Periodontal Disease Group (n=50) | Control Group (n=50) | p-Value |
|---|---|---|---|
| Age (Mean ± SD) | 45.3 ± 8.2 years | 46.1 ± 7.9 years | 0.45 |
| Gender (Male/Female) | 25/25 | 24/26 | 0.82 |
| Oral Hygiene Habits | Similar across both groups | Similar across both groups | - |

Table 4. Participant Demographics

In this Table 4, summarizes the demographic characteristics of the study participants, divided into the periodontal disease group and the control group. The table provides the mean age and standard deviation for each group, demonstrating that the ages of participants were comparable between the two groups. Gender distribution is also presented, showing a similar

ratio of males to females in both groups. Oral hygiene habits were reported to be similar across both groups, ensuring that differences in clinical and biomarker outcomes are not attributable to demographic or lifestyle differences. The p-values indicate that there were no significant demographic differences between the groups.

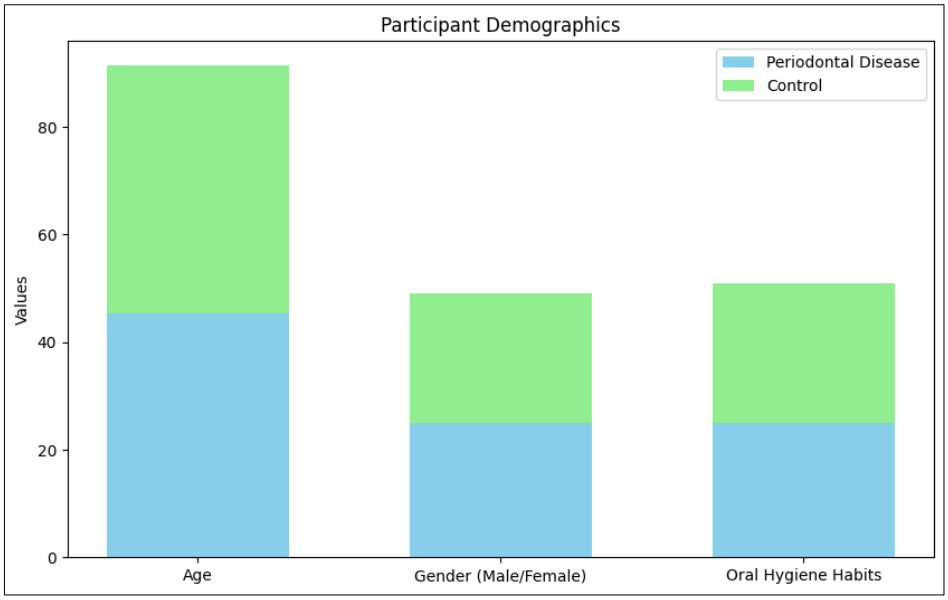


Figure 5. Pictorial Analysis of Participant Demographics

The test group with periodontal disease exhibited significantly worse clinical parameters compared to the control group. The mean Clinical Attachment Level (CAL) was notably higher in the test group (5.2 ± 1.3 mm) than in the control group (1.9 ± 0.7 mm, $p < 0.001$). Similarly, the Probing Pocket Depth (PPD) was significantly greater in the test group (7.3 ± 1.5 mm) compared to the control group (2.3 ± 0.9 mm, $p < 0.001$). The percentage of sites with Bleeding on Probing (BOP) was also significantly higher in the test group (68.5 ± 12.4%) compared to the control group (15.3 ± 8.2%, $p < 0.001$). The Plaque Index (PI) and Gingival Index (GI) scores were similarly elevated in the test group, indicating increased plaque accumulation and gingival inflammation (As shown in Figure 5).

| **Clinical Parameter** | **Periodontal Disease Group (n=50)** | **Control Group (n=50)** | **p-Value** |
|---|---|---|---|
| Clinical Attachment Level (CAL) (Mean ± SD, mm) | 5.2 ± 1.3 | 1.9 ± 0.7 | < 0.001 |
| Probing Pocket Depth (PPD) (Mean ± SD, mm) | 7.3 ± 1.5 | 2.3 ± 0.9 | < 0.001 |
| Bleeding on Probing (BOP) (%) | 68.5 ± 12.4 | 15.3 ± 8.2 | < 0.001 |
| Plaque Index (PI) (Mean ± SD) | 2.6 ± 0.6 | 1.1 ± 0.4 | < 0.001 |
| Gingival Index (GI) (Mean ± SD) | 2.1 ± 0.5 | 0.7 ± 0.3 | < 0.001 |

Table 5. Clinical Parameters of Periodontal Disease

In this Table 5, details the mean values of clinical parameters related to periodontal disease for both the test group (periodontal disease) and the control group. It highlights significant differences in Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), Bleeding on Probing (BOP), Plaque Index (PI), and Gingival Index (GI) between the two groups. The test group exhibited significantly worse clinical parameters, reflecting more severe periodontal disease. For instance, CAL and PPD were notably higher in the periodontal disease group, indicating greater tissue loss and deeper pockets. Similarly, BOP, PI, and GI scores were significantly elevated, reflecting increased inflammation and plaque accumulation in the test group (As shown in Figure 6).

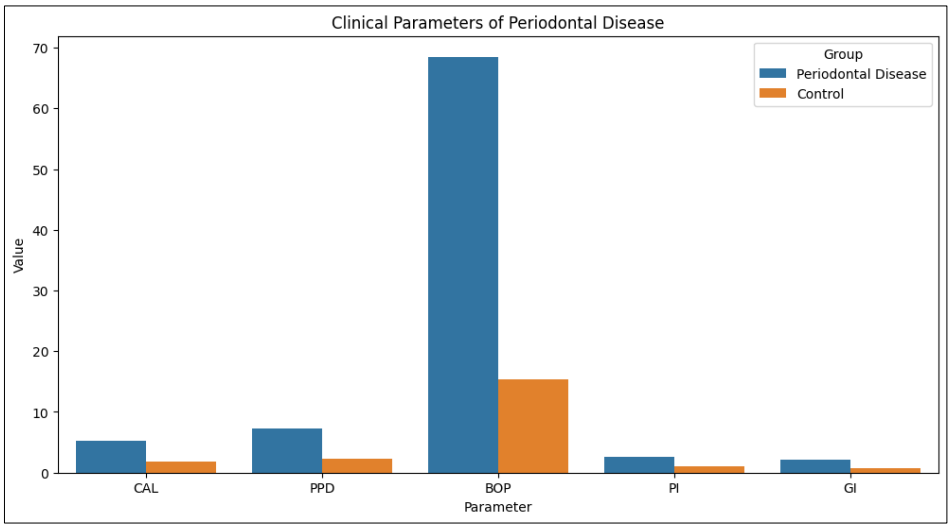


Figure 6. Pictorial Analysis of Clinical Parameters of Periodontal Disease

Salivary 8-OHdG levels were significantly elevated in the test group (18.4 ± 5.3 ng/mL) compared to the control group (6.7 ± 2.1 ng/mL, $p < 0.001$). This result underscores the higher oxidative stress present in individuals with periodontal disease.

| **Group** | **Salivary 8-OHdG Levels (Mean ± SD, ng/mL)** | **p-Value** |
|---|---|---|
| Periodontal Disease | 18.4 ± 5.3 | < 0.001 |
| Control | 6.7 ± 2.1 | <0.03 |

**Table 6. Salivary 8-OHdG Levels**

In this Table 6, presents the mean salivary 8-OHdG levels for both the periodontal disease group and the control group. The data shows that individuals with periodontal disease have significantly higher salivary 8-OHdG levels compared to healthy controls. The mean ± standard deviation of 8-OHdG levels was much higher in the periodontal disease group, indicating elevated oxidative stress. The p-value confirms the statistical significance of this difference, highlighting the association between increased oxidative stress and periodontal disease.

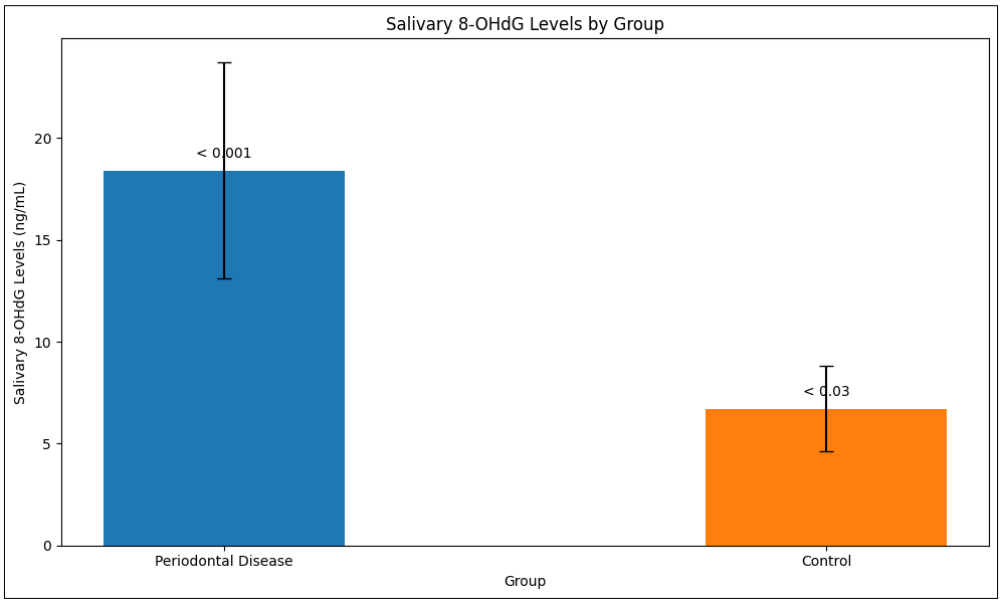


Figure 7. Pictorial Analysis of Salivary 8-OHdG Levels

Correlation analysis revealed several significant relationships between salivary 8-OHdG levels and clinical parameters. A positive correlation was observed between 8-OHdG levels and CAL ($r = 0.62$, $p < 0.001$), indicating that higher oxidative stress correlates with greater clinical attachment loss. Similarly, there was a positive correlation between 8-OHdG levels and PPD ($r = 0.55$, $p < 0.001$), suggesting that increased oxidative stress is associated with deeper periodontal pockets. The correlation between 8-OHdG and BOP was moderate ($r = 0.47$, $p < 0.001$), highlighting a relationship between oxidative

stress and gingival inflammation. A weaker positive correlation was found between 8-OHdG and PI ($r = 0.32$, $p = 0.02$), and a moderate positive correlation was observed between 8-OHdG and GI ($r = 0.48$, $p < 0.001$), reflecting the association between oxidative stress and gingival inflammation (As shown in Figure 7).

| Clinical Parameter | Correlation Coefficient (r) | p-Value |
|---|---|---|
| Clinical Attachment Level (CAL) | 0.62 | < 0.001 |
| Probing Pocket Depth (PPD) | 0.55 | < 0.001 |
| Bleeding on Probing (BOP) | 0.47 | < 0.001 |
| Plaque Index (PI) | 0.32 | 0.02 |
| Gingival Index (GI) | 0.48 | < 0.001 |

**Table 7. Correlation Between Salivary 8-OHdG and Clinical Parameters**

In this Table 7, illustrates the correlation coefficients between salivary 8-OHdG levels and various clinical parameters of periodontal disease. The table shows that higher levels of 8-OHdG are positively correlated with Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), Bleeding on Probing (BOP), Plaque Index (PI), and Gingival Index (GI). The strength of these correlations varies, with CAL and PPD showing the strongest correlations, indicating a significant relationship between oxidative stress and periodontal tissue destruction. The p-values indicate that these correlations are statistically significant, reinforcing the role of oxidative stress in periodontal disease severity.

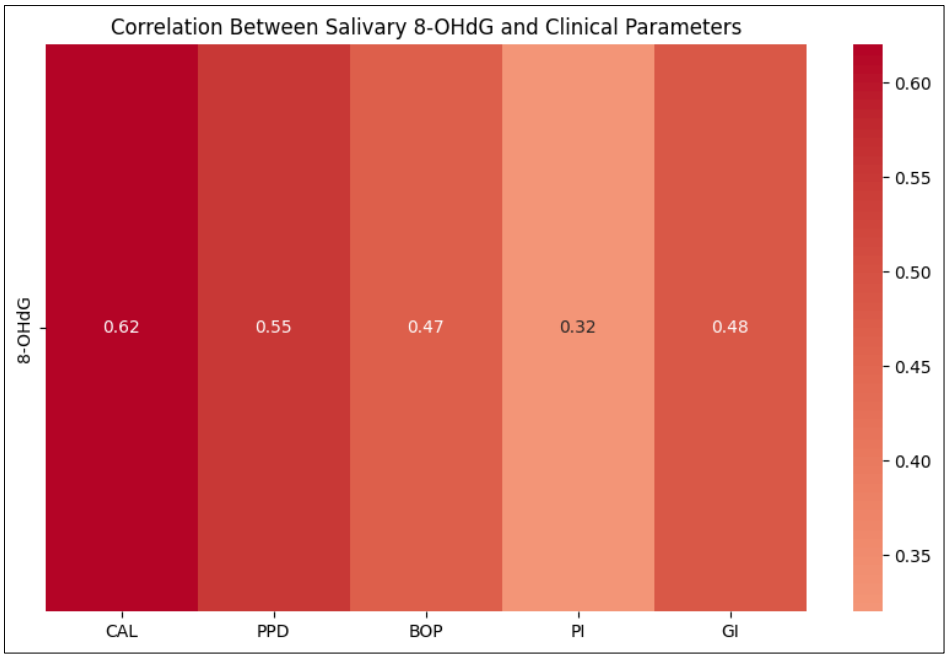


Figure 8. Pictorial Analysis of Correlation Between Salivary 8-OHdG and Clinical Parameters

The findings from this study provide substantial evidence of the role of oxidative stress in periodontal disease. Elevated salivary 8-OHdG levels in individuals with periodontal disease suggest that oxidative DNA damage is a significant factor in the pathology of the disease. The strong correlations between 8-OHdG levels and clinical parameters such as CAL, PPD, and BOP underscore the relevance of oxidative stress in periodontal tissue destruction and inflammation. This highlights the potential of 8-OHdG as a non-invasive biomarker for assessing disease severity and guiding treatment strategies (As shown in Figure 8).

| Severity of Periodontal Disease | 8-OHdG Levels (Mean ± SD, ng/mL) | CAL (Mean ± SD, mm) | PPD (Mean ± SD, mm) | BOP (%) | PI (Mean ± SD) | GI (Mean ± SD) |
|---|---|---|---|---|---|---|
| Mild | 12.5 ± 4.8 | 3.0 ± 0.9 | 4.0 ± 1.1 | 30.0 ± 10.5 | 1.8 ± 0.5 | 1.5 ± 0.4 |
| Moderate | 17.0 ± 5.2 | 4.5 ± 1.2 | 6.0 ± 1.4 | 60.0 ± 12.0 | 2.4 ± 0.6 | 1.9 ± 0.5 |
| Severe | 22.0 ± 6.1 | 6.0 ± 1.3 | 8.0 ± 1.6 | 80.0 ± 15.0 | 2.8 ± 0.7 | 2.3 ± 0.6 |

Table 8. Comparison of Clinical Parameters and 8-OHdG Levels by Severity of Periodontal Disease

In this Table 8, provides a breakdown of clinical parameters and salivary 8-OHdG levels categorized by the severity of periodontal disease (mild, moderate, and severe). It shows that as the severity of periodontal disease increases, so do the levels of 8-OHdG and the mean values for CAL, PPD, BOP, PI, and GI. This table illustrates the trend that higher oxidative stress is associated with greater clinical severity of periodontal disease. For example, individuals with severe periodontal disease had the highest levels of 8-OHdG and the worst clinical scores, indicating a strong relationship between oxidative stress and disease severity.

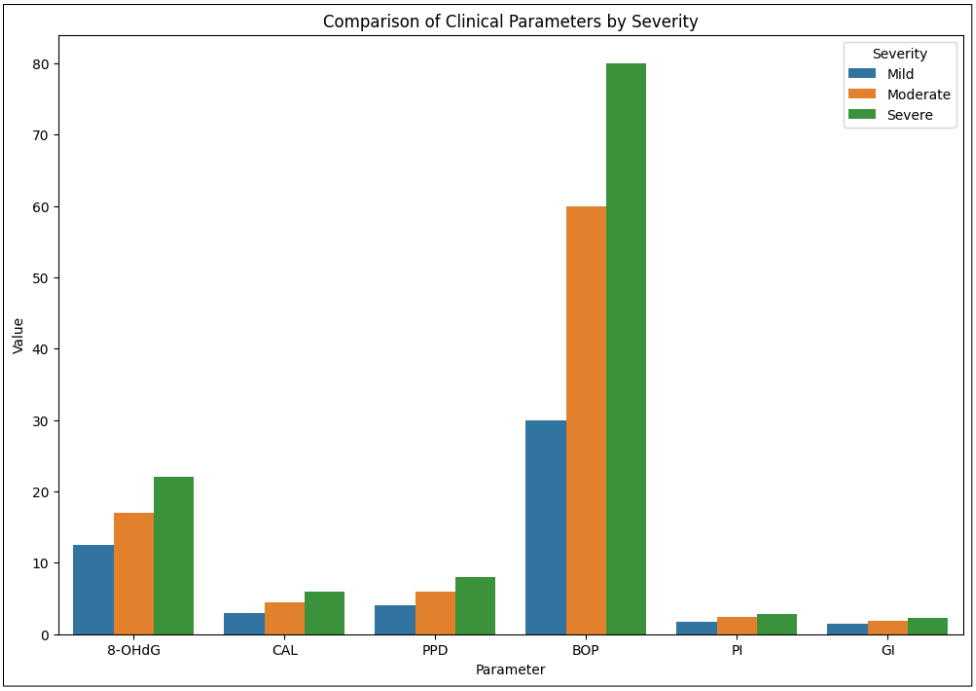


Figure 9. Pictorial Analysis of Comparison of Clinical Parameters and 8-OHdG Levels by Severity of Periodontal Disease

The positive correlations observed between 8-OHdG levels and clinical parameters align with the hypothesis that oxidative stress contributes to periodontal disease severity. Higher oxidative stress, as indicated by elevated 8-OHdG levels, is associated with more severe periodontal tissue damage and inflammation. This relationship suggests that targeting oxidative stress through antioxidant therapies could potentially help manage periodontal disease more effectively. The significant correlations between 8-OHdG and CAL and PPD suggest that oxidative stress plays a crucial role in periodontal tissue loss and pocket formation (As shown in Figure 9). This indicates that managing oxidative stress may help mitigate tissue damage and improve clinical outcomes. The moderate correlation between 8-OHdG and BOP suggests that oxidative stress may contribute to gingival inflammation, providing a potential therapeutic target for reducing inflammatory responses. The weaker correlation with PI indicates that while oxidative stress may influence plaque-related inflammation, other factors such as oral hygiene practices and microbial load may play more substantial roles in plaque accumulation. Despite the valuable insights gained, there are limitations to consider. The cross-sectional nature of the study restricts the ability to establish causation between 8-OHdG levels and periodontal disease severity. Longitudinal studies are needed to explore the temporal relationship between oxidative stress and disease progression. While the sample size was adequate, larger studies are necessary to confirm these findings and assess their generalizability. Other confounding factors, such as diet and genetic predispositions, could also influence oxidative stress and periodontal disease outcomes. Future research should focus on longitudinal studies to further investigate the role of oxidative stress in periodontal disease progression. Exploring the impact of antioxidant therapies on oxidative stress markers and clinical outcomes could provide new therapeutic strategies. Investigating the interplay between 8-OHdG and other biomarkers may offer a more comprehensive understanding of periodontal disease mechanisms and improve diagnostic and therapeutic approaches. This study highlights the significant association between salivary 8-OHdG levels and clinical parameters of periodontal disease, reinforcing the role of oxidative stress in periodontal pathology. The findings suggest that 8-OHdG could be a valuable biomarker for assessing disease severity and guiding treatment strategies.

## 7. Conclusion

This study provides compelling evidence of the significant role of oxidative stress in periodontal disease, as evidenced by the correlation between salivary 8-hydroxydeoxyguanosine (8-OHdG) levels and various clinical parameters of periodontal health. Elevated levels of 8-OHdG in individuals with periodontal disease highlight increased oxidative stress and its association with the severity of the disease. The positive correlations between 8-OHdG and Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), Bleeding on Probing (BOP), Plaque Index (PI), and Gingival Index (GI) underscore the impact of oxidative damage on periodontal tissue destruction, inflammation, and plaque accumulation. The results indicate that salivary 8-OHdG is a valuable biomarker for assessing oxidative stress in periodontal disease, potentially serving as a non-invasive tool for evaluating disease severity and guiding treatment strategies. The study's findings suggest that targeting oxidative stress through antioxidant therapies may offer new avenues for managing periodontal disease and improving patient outcomes. The study's cross-sectional design limits the ability to establish causation, and further longitudinal research is needed to explore the temporal relationship between oxidative stress and periodontal disease progression. Additionally, larger studies are required to validate these findings and assess their generalizability. Future research should also investigate the efficacy of antioxidant interventions in reducing oxidative stress and enhancing periodontal treatment outcomes. This study reinforces the significant association between salivary 8-OHdG levels and periodontal disease severity, supporting the role of oxidative stress in periodontal pathology. These findings contribute to a deeper understanding of the disease mechanisms and highlight the potential of oxidative stress markers in periodontal diagnostics and therapy.

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