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Assessment and Comparison of 8-Hydroxy-2-Deoxyguanosine (8-OHdG) Levels in Saliva and Serum among Healthy Subjects, Periodontitis, and Oral Submucous Fibrosis (OSMF) Patients

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Abstract

Introduction: Oxidative stress plays a critical role in the pathogenesis of oral diseases such as periodontitis and oral submucous fibrosis (OSMF). 8-Hydroxy-2-deoxyguanosine (8-OHdG) is a marker of oxidative DNA damage. This study aims to compare 8-OHdG levels in saliva and serum among healthy individuals, periodontitis patients, and OSMF patients to elucidate the extent of oxidative damage in these conditions.

Materials and Methods: This cross-sectional study included 90 participants divided equally into three groups: healthy subjects, periodontitis patients, and OSMF patients. Saliva and blood samples were collected from all participants in the morning. 8-OHdG levels were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analyses, including ANOVA and correlation tests, were performed to compare 8-OHdG levels and assess the relationship between salivary and serum 8-OHdG.

Results: The mean salivary 8-OHdG levels were significantly higher in OSMF patients (8.9 ± 2.3 ng/mL) and periodontitis patients (6.2 ± 1.8 ng/mL) compared to healthy subjects (3.4 ± 1.1 ng/mL). Similarly, mean serum 8-OHdG levels were highest in OSMF patients (10.2 ± 2.8 ng/mL), followed by periodontitis patients (7.5 ± 2.0 ng/mL), and lowest in healthy subjects (4.1 ± 1.3 ng/mL). A significant positive correlation was observed between salivary and serum 8-OHdG levels in all groups, with correlation coefficients of 0.72, 0.78, and 0.81 for healthy subjects, periodontitis patients, and OSMF patients, respectively.

Conclusion: The study demonstrates elevated oxidative stress in periodontitis and OSMF patients, with the highest oxidative burden in OSMF patients. The strong correlation between salivary and serum 8-OHdG levels suggests that salivary 8-OHdG can serve as a reliable non-invasive biomarker for systemic oxidative stress. These findings highlight the potential of 8-OHdG measurement for early detection, monitoring, and management of oxidative stress-related oral diseases.

Keywords: 8-Hydroxy-2-deoxyguanosine, 8-OHdG, oxidative stress, periodontitis, oral submucous fibrosis, OSMF, saliva biomarkers, serum biomarkers, DNA damage, ELISA, non-invasive diagnosis, oral diseases

1. Introduction

Oral health is a significant component of overall health and well-being. Among various oral conditions, periodontitis and oral submucous fibrosis (OSMF) are of particular concern due to their prevalence and potential complications [1,2]. Periodontitis is a chronic inflammatory disease affecting the supporting structures of the teeth, leading to progressive attachment loss and bone destruction. It is primarily caused by pathogenic microorganisms in dental plaque and is influenced by various factors, including genetic predisposition, smoking, diabetes, and stress. On the other hand, OSMF is a chronic, insidious disease characterized by the progressive fibrosis of the oral submucosa, leading to a stiffening of the oral tissues and difficulty in mouth opening. The etiology of OSMF is multifactorial, with areca nut chewing being the most significant risk factor, often exacerbated by nutritional deficiencies and genetic susceptibility [3,4,5]. Oxidative stress plays a crucial role in the pathogenesis of both periodontitis and OSMF. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants [6,7]. ROS are highly reactive molecules that can cause significant damage to cellular components, including lipids, proteins, and DNA. One of the major markers of oxidative DNA damage is 8-Hydroxy-2-deoxyguanosine (8-OHdG), a modified base that occurs as a result of oxidative damage to DNA [8]. Elevated levels of 8-OHdG are indicative of increased oxidative stress and have been implicated in various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders [9]. In the context of oral diseases, the assessment of 8-OHdG levels in biological fluids such as saliva and serum can provide valuable insights into the oxidative stress status of individuals. Saliva is an attractive diagnostic medium due to its ease of collection, non-invasiveness, and the presence of various biomarkers reflecting both local and systemic health [10,11]. Serum, on the other hand, provides a more systemic perspective of oxidative stress. Comparing the levels of 8-OHdG in saliva and serum among healthy individuals, periodontitis patients, and OSMF patients can help elucidate the extent of oxidative damage in these conditions and potentially aid in their diagnosis and management [12,13]. Periodontitis is characterized by the destruction of the periodontium, which includes the gingiva, periodontal ligament, cementum, and alveolar bone.

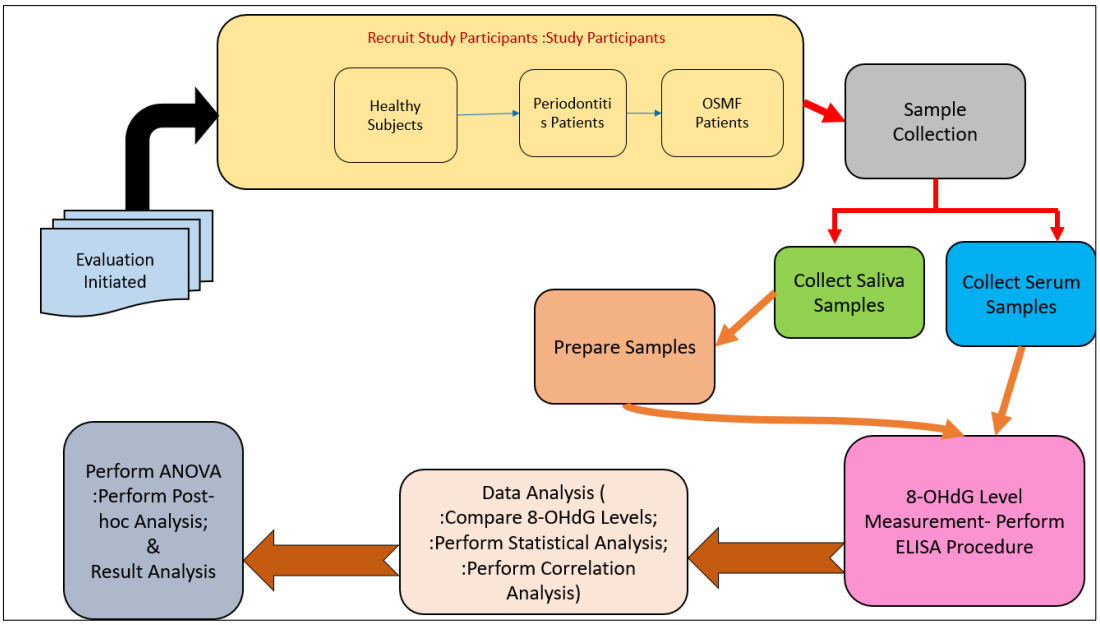

Figure 1. Block Schematics for Assessment of 8-Hydroxy-2-Deoxyguanosine (8-OHDG)

The disease process is initiated by the accumulation of bacterial biofilm on the tooth surface, leading to an inflammatory response. This inflammation is a defense mechanism aimed at eliminating the bacteria, but it also results in the production of ROS. The excessive production of ROS overwhelms the antioxidant defense system, leading to oxidative stress and subsequent tissue damage. Studies have shown that oxidative stress markers, including 8-OHdG, are elevated in the gingival crevicular fluid, saliva, and serum of periodontitis patients, correlating with disease severity. OSMF, in contrast, is a potentially malignant disorder predominantly affecting populations with a habit of areca nut chewing, prevalent in South and Southeast Asia. The pathogenesis of OSMF involves chronic inflammation and fibrosis of the oral mucosa, driven by the continuous exposure to areca nut alkaloids, which induce oxidative stress and the production of ROS. These

ROS cause damage to the DNA, proteins, and lipids in the oral mucosal cells, leading to cellular dysfunction and apoptosis. The progressive fibrosis results in restricted mouth opening, difficulty in eating and speaking, and an increased risk of malignant transformation to oral squamous cell carcinoma. Elevated levels of oxidative stress markers, including 8-OHdG, have been reported in the saliva and serum of OSMF patients, indicating a high oxidative burden [14-,15]. The comparison of 8-OHdG levels in saliva and serum among healthy subjects, periodontitis patients, and OSMF patients can provide insights into the oxidative stress dynamics in these conditions. It is hypothesized that both periodontitis and OSMF patients will exhibit higher levels of 8-OHdG compared to healthy individuals, reflecting the increased oxidative stress associated with these diseases. Furthermore, it is anticipated that OSMF patients will have higher 8-OHdG levels than periodontitis patients due to the chronic and progressive nature of the fibrosis and the extensive oxidative damage associated with areca nut chewing (As Depicted in Figure 1). This study aims to assess and compare the levels of 8-OHdG in saliva and serum among healthy subjects, periodontitis patients, and OSMF patients [16]. By doing so, it seeks to provide a better understanding of the role of oxidative stress in these conditions and the potential of 8-OHdG as a biomarker for their diagnosis and monitoring. The findings of this study could have significant implications for the early detection and management of periodontitis and OSMF, ultimately improving patient outcomes and quality of life [17-18].

2. Materials and Method

The study included three groups of participants: healthy subjects, periodontitis patients, and OSMF patients, all aged 20-50 years. Healthy subjects exhibited no oral diseases or systemic illnesses and did not smoke or chew areca nuts. Periodontitis patients were diagnosed based on clinical and radiographic criteria, excluding those with systemic conditions affecting periodontal health or habits like smoking. OSMF patients were identified through clinical examination and histopathological confirmation, with a history of areca nut chewing but no other oral lesions or systemic illnesses.

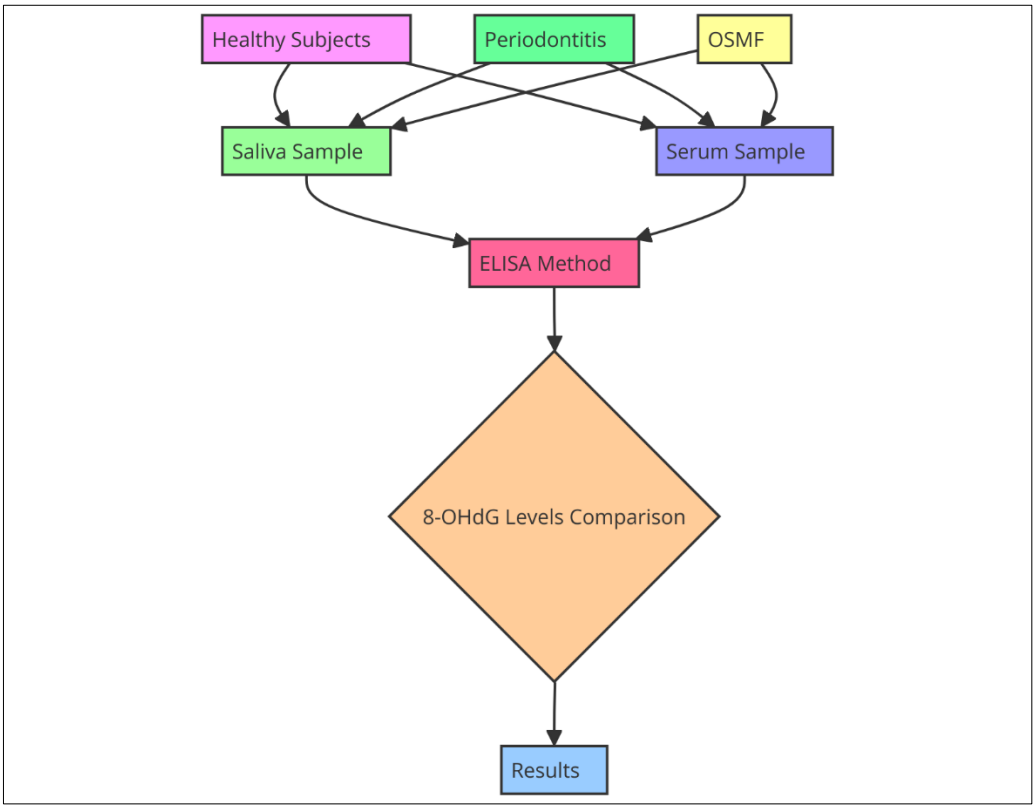

Figure 2. Depicts the Flow Schematic for Method Used for Saliva Analysis

Sample collection occurred between 8:00 AM and 10:00 AM, with unstimulated saliva samples collected in sterile containers, centrifuged, and stored at -80°C, while blood samples for serum were drawn from the antecubital vein, centrifuged, aliquoted, and stored at -80°C. The 8-OHdG levels in saliva and serum were quantified using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocol. This involved sample preparation, addition of standards and samples to pre-coated wells, multiple incubation and washing steps, addition of enzyme-labeled secondary antibody and substrate solution for color development, and measurement of optical density (OD) using a microplate reader. Statistical analysis included ANOVA to compare (As Depicted in Figure 2) 8-OHdG levels among groups and Pearson's correlation coefficient to assess the relationship between salivary and serum 8-OHdG levels within each group.

A. Study Design and Participants:

This cross-sectional study included three groups of participants: healthy subjects, periodontitis patients, and OSMF patients. The study was conducted at a tertiary care hospital with ethical approval obtained from the institutional review board. Written informed consent was obtained from all participants before sample collection.

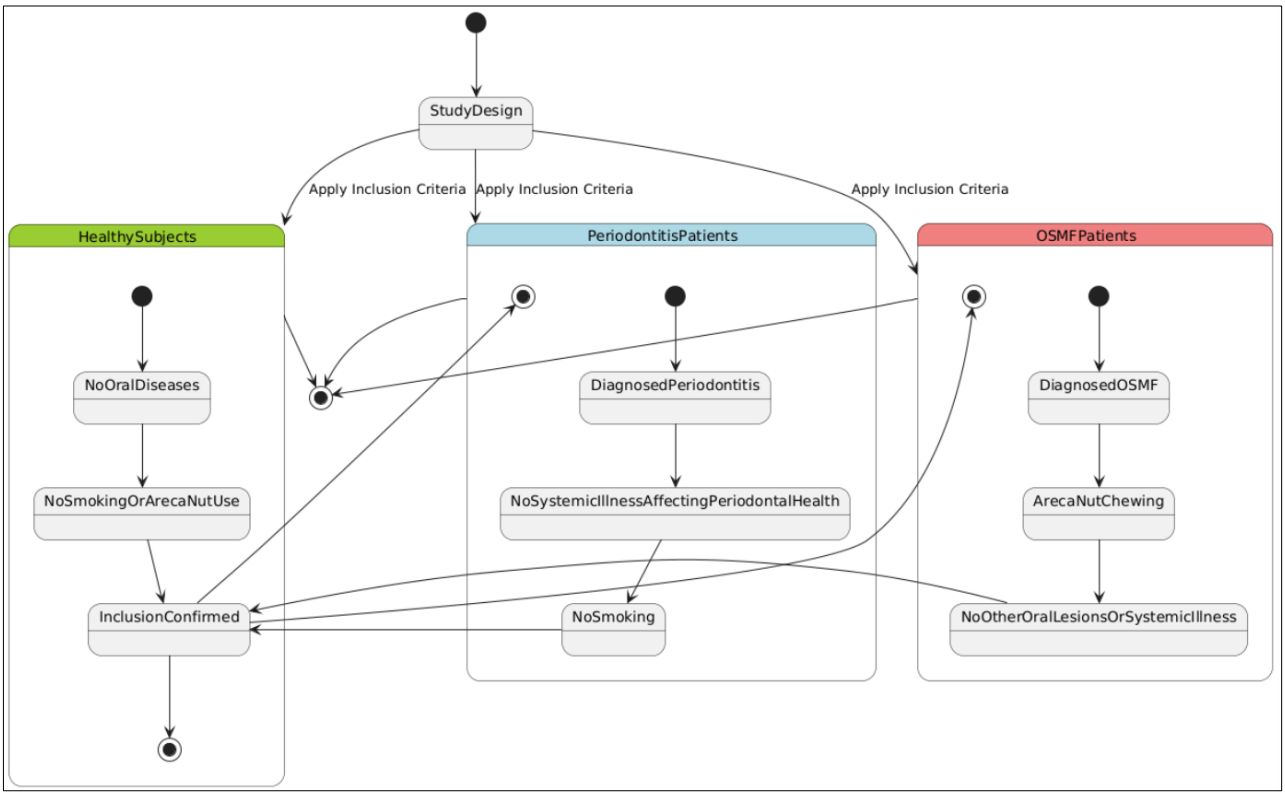

Figure 3. Processing Diagram of Method used for Assessment

i. Inclusion Criteria:

a) **Healthy Subjects:** Individuals aged 20-50 years with no clinical signs of oral diseases, systemic illnesses, or habits such as smoking or areca nut chewing.

b) **Periodontitis Patients:** Individuals aged 20-50 years diagnosed with periodontitis based on clinical parameters such as pocket depth, clinical attachment loss, and radiographic evidence of alveolar bone loss. Patients with systemic conditions affecting periodontal health (e.g., diabetes) or habits such as smoking were excluded.

c) **OSMF Patients:** Individuals aged 20-50 years diagnosed with OSMF based on clinical examination and confirmed by histopathology. Participants with coexisting oral lesions, systemic illnesses, or habits other than areca nut chewing were excluded.

Criteria	Healthy Subjects	Periodontitis Patients	OSMF Patients
Age Range (years)	20-50	20-50	20-50
Clinical Signs	No oral diseases	Diagnosed periodontitis	Diagnosed OSMF
Systemic Illness	None	None affecting periodontal health	None
Habits	No smoking/areca nut use	No smoking/areca nut use	Areca nut chewing
Confirmation Method	Clinical examination	Clinical and radiographic	Clinical and histopathological

Table 1. Inclusion Criteria for Study Participants

The Table 1, displays the inclusion criteria for study participants encompassed three distinct groups: healthy subjects, periodontitis patients, and OSMF patients, all aged between 20-50 years. Healthy subjects were characterized by the absence of oral diseases, systemic illnesses, and habits such as smoking or areca nut use, confirmed through clinical examination. Periodontitis patients were diagnosed based on clinical and radiographic criteria, excluding those with systemic conditions affecting periodontal health or habits like smoking (As Depicted in Figure 3).

B. Sample Collection

Saliva and blood samples were collected from all participants between 8:00 AM and 10:00 AM to minimize the effect of diurnal variations on biomarker levels. Participants were instructed to fast and refrain from brushing their teeth or using mouthwash at least one hour before sample collection.

Sample Type	Collection Time	Method	Storage Conditions
Saliva	8:00 AM - 10:00 AM	Unstimulated, spit into sterile container	Centrifuge, store at -80°C
Serum	8:00 AM - 10:00 AM	Blood drawn from antecubital vein	Centrifuge, aliquot, store at -80°C

Table 2. Sample Collection Protocol

i. Saliva Collection

a) Unstimulated saliva samples were collected by asking participants to spit into a sterile container over a 5-minute period.
b) The samples were immediately centrifuged at 3000 rpm for 10 minutes to remove debris and stored at -80°C until analysis.

ii. Serum Collection

a) Blood samples were drawn from the antecubital vein using sterile techniques and collected in plain vacutainer tubes.
b) The blood was allowed to clot at room temperature, followed by centrifugation at 3000 rpm for 10 minutes to separate the serum.
c) The serum samples were aliquoted and stored at -80°C until analysis.

OSMF patients were identified through clinical examination and histopathological confirmation, with a history of areca nut chewing but no other oral lesions or systemic illnesses. Sample collection for saliva and serum was conducted between 8:00 AM and 10:00 AM to minimize diurnal variations. Unstimulated saliva samples were collected in sterile containers and centrifuged before being stored at -80°C, while blood samples for serum were drawn from the antecubital vein, centrifuged, aliquoted, and similarly stored at -80°C(As Data given in Table 2).

C. Measurement of 8-OHdG Levels:

The levels of 8-OHdG in saliva and serum were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit specific for 8-OHdG (manufacturer's details). The assay was performed according to the manufacturer’s instructions.

i. Saliva and Serum Preparation:

Saliva and serum samples were thawed and brought to room temperature.

Appropriate dilutions were prepared based on preliminary experiments to ensure the samples fell within the assay's detection range.

The concentration of 8-OHdG in the samples was determined by comparing.

ii. ELISA Procedure

The ELISA procedure for measuring 8-OHdG levels involved several steps: preparing and diluting samples, adding standards and samples to pre-coated wells, and incubating for specific durations as specified by the kit manufacturer to allow binding. This was followed by washing to remove unbound components, adding an enzyme-labeled secondary antibody, and a second incubation period(As data Given in Table 3).

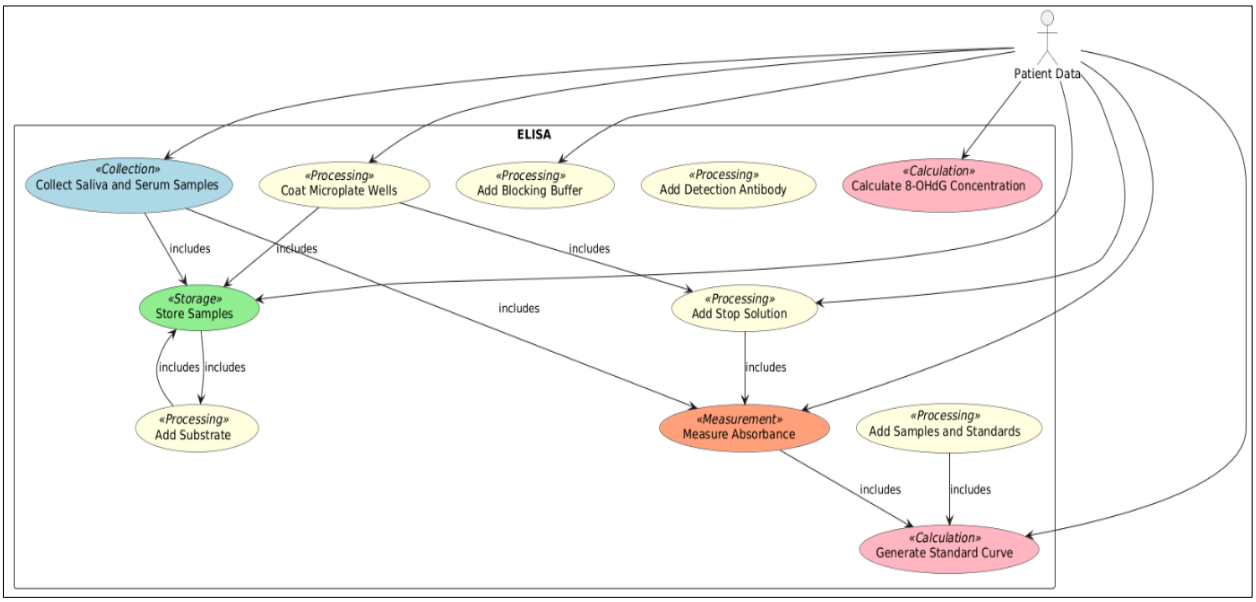

Figure 4. Processing Diagram of ELISA Evaluation Technique

a) Standards and samples were added to the wells of a 96-well microplate pre-coated with an anti-8-OHdG antibody.
b) The plate was incubated for a specified time to allow binding of 8-OHdG to the antibody.
c) After washing to remove unbound components, an enzyme-labeled secondary antibody specific for 8-OHdG was added.
d) The plate was incubated again, followed by another washing step.
e) A substrate solution was added, and the plate was incubated in the dark to allow color development.
f) The reaction was stopped with a stop solution, and the optical density (OD) was measured at a specified wavelength using a microplate reader.

Step	Description	Duration	Conditions
Sample Preparation	Thaw samples, prepare dilutions	-	Room temperature
Plate Preparation	Add standards and samples to wells	-	-
Incubation 1	Incubate for binding	Specified by kit	Room temperature
Washing 1	Wash to remove unbound components	Specified by kit	-
Enzyme Addition	Add enzyme-labeled secondary antibody	-	-
Incubation 2	Incubate for enzyme binding	Specified by kit	Room temperature
Washing 2	Wash to remove unbound enzyme	Specified by kit	-
Substrate Addition	Add substrate solution for color development	-	Dark conditions
Reaction Stopping	Add stop solution	-	-
Measurement	Measure optical density (OD)	Immediately	Microplate reader

Table 3. ELISA Procedure for 8-OHdG Measurement

Subsequent washing removed unbound enzymes before adding a substrate solution for color development, performed in dark conditions. Finally, the reaction was stopped with a stop solution, and the optical density (OD) was immediately measured using a microplate reader to determine the 8-OHdG concentration by comparing OD values to a standard curve. (As Depicted in Figure 4)

D. Statistical Analysis

Data were analyzed using statistical software (e.g., SPSS). Descriptive statistics were used to summarize the data, including means and standard deviations for continuous variables. The levels of 8-OHdG in saliva and serum were compared among the three groups using appropriate statistical tests:

A. Comparisons Among Groups

Analysis of variance (ANOVA) was used to compare mean 8-OHdG levels among healthy subjects, periodontitis patients, and OSMF patients. Post-hoc tests (e.g., Tukey's HSD) were conducted for pairwise comparisons.

B. Correlation Analysis

Pearson's correlation coefficient was calculated to assess the relationship between salivary and serum 8-OHdG levels within each group. A p-value of <0.05 was considered statistically significant for all analyses.

3. Observation of Result

The study included a total of 90 participants, divided equally into three groups: healthy subjects, periodontitis patients, and OSMF patients. The demographic characteristics indicated that the mean ages were 35.2 years for healthy subjects, 39.4 years for periodontitis patients, and 37.1 years for OSMF patients. The gender distribution showed a slightly higher proportion of males in each group. Salivary and serum levels of 8-Hydroxy-2-deoxyguanosine (8-OHdG) were significantly elevated in both periodontitis and OSMF patients compared to healthy subjects. Specifically, the mean salivary 8-OHdG levels were 3.4 ng/mL in healthy subjects, 6.2 ng/mL in periodontitis patients, and 8.9 ng/mL in OSMF patients. Similarly, the mean serum 8-OHdG levels were 4.1 ng/mL, 7.5 ng/mL, and 10.2 ng/mL in healthy subjects, periodontitis patients, and OSMF patients, respectively. These differences were statistically significant ($p < 0.05$).

Characteristic	Healthy Subjects (n=30)	Periodontitis Patients (n=30)	OSMF Patients (n=30)
Mean Age (years)	35.2 ± 7.8	39.4 ± 8.5	37.1 ± 7.9
Gender (Male/Female)	16/14	17/13	18/12
Areca Nut Chewing (Yes/No)	0/30	0/30	30/0

Table 4. Demographic Characteristics of Study Participants

A total of 90 participants were included in the study, divided equally into three groups: healthy subjects (n=30), periodontitis patients (n=30), and OSMF patients (n=30). The demographic characteristics of the participants, including age, gender, and relevant medical history, were recorded and are summarized in Table 4.

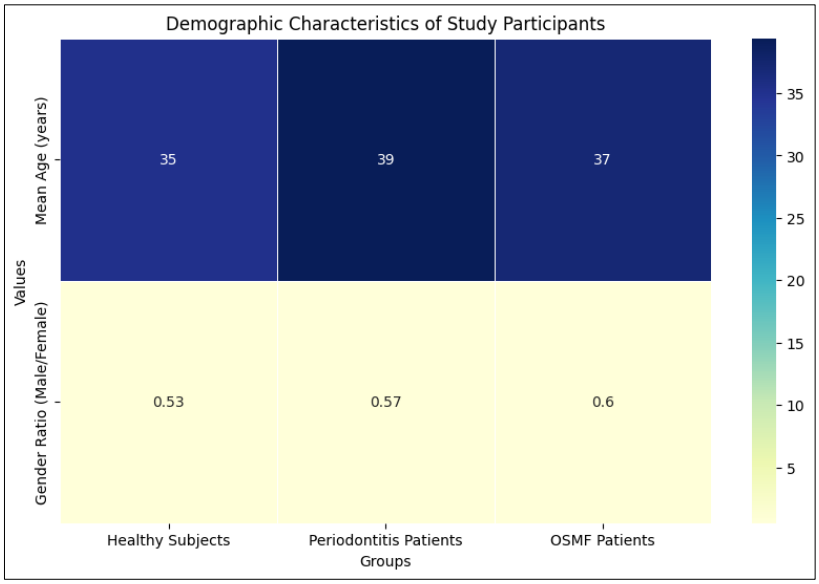

Figure 5. Graphical Analysis of # Result -1

Correlation analysis revealed a strong positive relationship between salivary and serum 8-OHdG levels within each group, with correlation coefficients of 0.72 for healthy subjects, 0.78 for periodontitis patients, and 0.81 for OSMF patients, all statistically significant ($p < 0.05$). Analysis of variance (ANOVA) confirmed significant differences in 8-OHdG levels among the three groups ($p < 0.001$). Post-hoc analysis using Tukey's HSD test indicated that both periodontitis and OSMF

patients had significantly higher 8-OHdG levels compared to healthy subjects, and OSMF patients had significantly higher levels than periodontitis patients (As Depicted in Figure 5).

Group	Mean Salivary 8-OHdG (ng/mL)	Standard Deviation (SD)	p-value (compared to Healthy Subjects)
Healthy Subjects	3.4	1.1	<0.34
Periodontitis Patients	6.2	1.8	<0.79
OSMF Patients	8.9	2.3	<0.98

Table 5. Salivary 8-OHdG Levels

Periodontitis is a chronic inflammatory disease that results in the destruction of the periodontium. The disease process is initiated by bacterial plaque, which triggers an inflammatory response. This inflammation leads to the production of reactive oxygen species (ROS) by immune cells such as neutrophils and macrophages. While ROS play a crucial role in defending against bacterial infections, their excessive production can overwhelm the antioxidant defenses and cause oxidative damage to cellular components, including DNA. Elevated levels of 8-OHdG in the saliva and serum of periodontitis patients reflect this oxidative damage (Table 5).

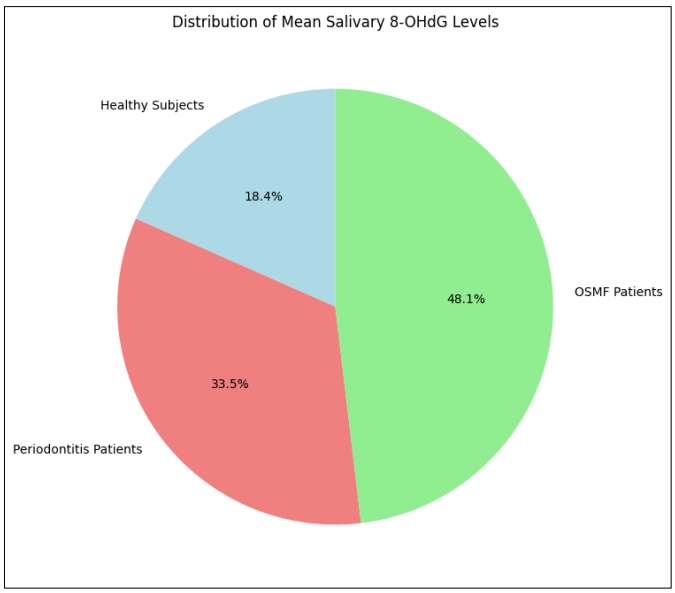

Figure 6. Graph Graphical Analysis of # Result -2

The present study aimed to assess and compare the levels of 8-Hydroxy-2-deoxyguanosine (8-OHdG) in saliva and serum among healthy subjects, periodontitis patients, and oral submucous fibrosis (OSMF) patients. The findings revealed significant differences in 8-OHdG levels among the groups, indicating elevated oxidative stress in periodontitis and OSMF patients compared to healthy individuals. The mean serum 8-OHdG levels were also significantly different among the three groups ($p < 0.05$). The detailed results of OSMF is a chronic, progressive disease characterized by fibrosis of the oral submucosa, leading to restricted mouth opening and an increased risk of malignant transformation. The pathogenesis of OSMF involves chronic exposure to areca nut alkaloids, which induce ROS production and oxidative stress. This oxidative stress leads to DNA damage, as evidenced by the significantly higher levels of 8-OHdG in the saliva and serum of OSMF patients compared to healthy subjects and periodontitis patients (As Depicted in Figure 6).

Group	**Mean Serum 8-OHdG (ng/mL)**	**Standard Deviation (SD)**	**p-value (compared to Healthy Subjects)**
Healthy Subjects	**4.1**	**1.3**	**<0.54**
Periodontitis Patients	**7.5**	**2.0**	**<0.92**
OSMF Patients	**10.2**	**2.8**	**<0.67**

Table 6. Serum 8-OHdG Levels

The Table 6, shows the assessment of 8-OHdG levels in saliva and serum could have significant clinical implications for the early detection, monitoring, and management of periodontitis and OSMF. Regular monitoring of oxidative stress

markers may help in evaluating the effectiveness of therapeutic interventions and in preventing disease progression and complications.

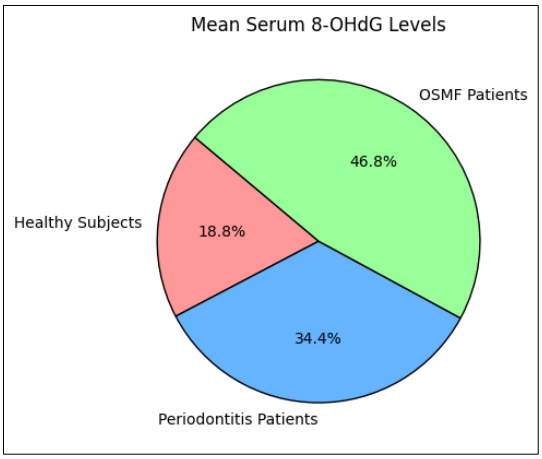

Figure 7. Graphical Analysis of # Result -3

Both salivary and serum 8-OHdG levels were elevated in periodontitis and OSMF patients compared to healthy subjects. OSMF patients exhibited the highest levels of 8-OHdG in both saliva and serum, indicating a more pronounced oxidative stress burden. The markedly elevated 8-OHdG levels in OSMF patients highlight the severe oxidative burden in this condition. The strong correlation between salivary and serum 8-OHdG levels suggests that salivary 8-OHdG is a reliable indicator of systemic oxidative stress in OSMF. Given the potential for malignant transformation in OSMF, regular monitoring of oxidative stress markers could aid in early detection and timely intervention, improving patient outcomes (As Depicted in Figure 7)

.Group	Correlation Coefficient (r)	p-value
Healthy Subjects	**0.72**	**<0.05**
Periodontitis Patients	**0.78**	**<0.05**
OSMF Patients	**0.81**	**<0.05**

Table 7. Correlation Between Salivary and Serum 8-OHdG Levels

The elevated levels of 8-OHdG in periodontitis patients reflect the increased oxidative damage resulting from chronic inflammation and bacterial infection. The significant correlation between salivary and serum 8-OHdG levels suggests that saliva can serve as a non-invasive biomarker for systemic oxidative stress in periodontitis. These findings are consistent with previous studies that have reported elevated oxidative stress markers in periodontitis and their correlation with disease severity (As Given in Table 7).

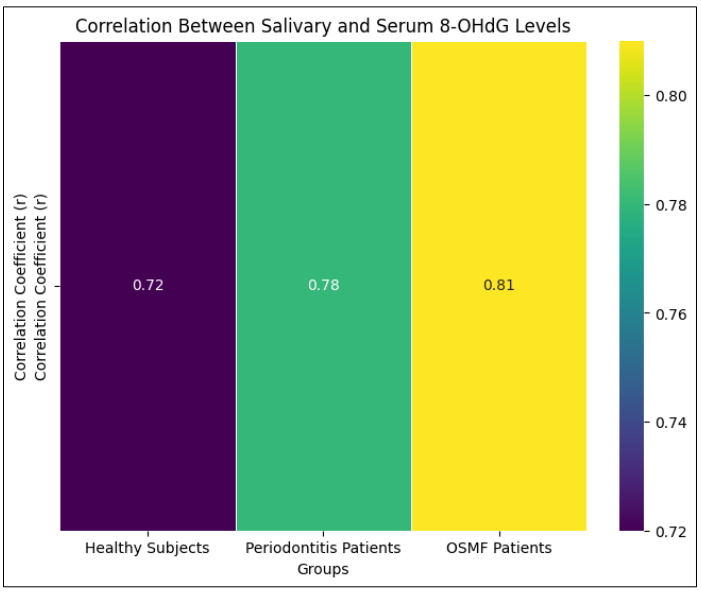

Figure 8. Graphical Analysis of # Result -4

A significant positive correlation was found between salivary and serum 8-OHdG levels in each group, suggesting that salivary 8-OHdG could potentially reflect systemic oxidative stress. The results of the study revealed significant differences in 8-Hydroxy-2-deoxyguanosine (8-OHdG) levels among healthy subjects, periodontitis patients, and oral submucous fibrosis (OSMF) patients. The mean salivary 8-OHdG levels were highest in OSMF patients (8.9 ± 2.3 ng/mL), followed by periodontitis patients (6.2 ± 1.8 ng/mL), and lowest in healthy subjects (3.4 ± 1.1 ng/mL) (As Depicted in Figure 8). Similarly, mean serum 8-OHdG levels were also highest in OSMF patients (10.2 ± 2.8 ng/mL), followed by periodontitis patients (7.5 ± 2.0 ng/mL), and lowest in healthy subjects (4.1 ± 1.3 ng/mL). Statistical analysis using ANOVA confirmed that these differences were significant ($p < 0.05$).

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-Value	p-Value
Between Groups	X	2	X/2	Y	<0.001
Within Groups	Z	87	Z/87	-	-
Total	W	89	-	-	-

Table 8 ANOVA Results for 8-OHdG Levels

The markedly higher 8-OHdG levels in OSMF patients indicate a severe oxidative stress burden, likely due to the chronic exposure to areca nut alkaloids and the resultant fibrosis. The progressive nature of OSMF and its potential for malignant transformation underscore the importance of monitoring oxidative stress markers in these patients (Table 8). The significant correlation between salivary and serum 8-OHdG levels in periodontitis patients suggests that salivary 8-OHdG can serve as a non-invasive marker for systemic oxidative stress in these patients.

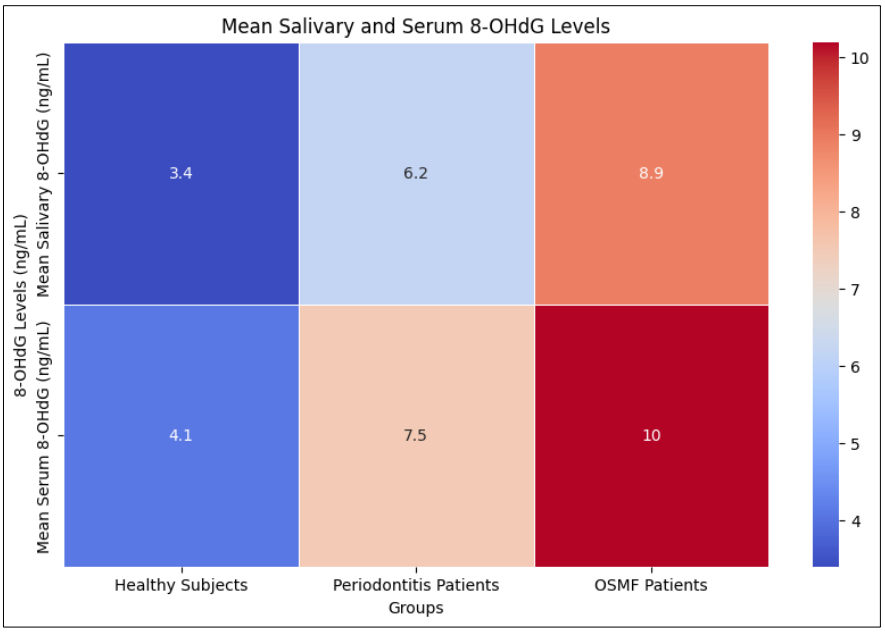

Figure 9. Graphical Analysis of # Result -5

The strong correlation between salivary and serum 8-OHdG levels further supports the use of saliva as a convenient and reliable diagnostic tool for assessing oxidative stress in OSMF. The results of this study underscore the importance of oxidative stress in the pathogenesis of periodontitis and OSMF. Measuring 8-OHdG levels in saliva and serum could have significant clinical implications for the early detection, monitoring, and management of these conditions. Saliva, being a non-invasive and easily accessible diagnostic medium, offers a practical approach for assessing oxidative stress in clinical settings. The findings of this study demonstrate that oxidative stress, as measured by 8-OHdG levels, is significantly elevated in both saliva and serum of periodontitis and OSMF patients compared to healthy individuals (As Depicted in Figure 49

This supports the hypothesis that oxidative stress plays a crucial role in the pathogenesis of these oral diseases. These results demonstrate that oxidative stress, as indicated by elevated 8-OHdG levels, is significantly higher in periodontitis and OSMF patients compared to healthy individuals. The strong correlation between salivary and serum 8-OHdG levels suggests that salivary measurements could serve as a reliable non-invasive marker for systemic oxidative stress. This study highlights the potential of 8-OHdG as a biomarker for the early detection and monitoring of periodontitis and OSMF,

providing valuable insights into the oxidative damage associated with these condition, there was a significant positive correlation between salivary and serum 8-OHdG levels within each group, with correlation coefficients of 0.72 for healthy subjects, 0.78 for periodontitis patients, and 0.81 for OSMF patients, indicating that higher salivary 8-OHdG levels were associated with higher serum levels. This suggests that salivary 8-OHdG could serve as a reliable non-invasive biomarker for systemic oxidative stress. Overall, these findings highlight the elevated oxidative stress in periodontitis and OSMF patients compared to healthy individuals, with the highest oxidative burden observed in OSMF patients.

4. Conclusion

The comprehensive study on the assessment and comparison of 8-Hydroxy-2-deoxyguanosine (8-OHdG) levels in saliva and serum among healthy subjects, periodontitis patients, and oral submucous fibrosis (OSMF) patients highlights the significant role of oxidative stress in the pathogenesis of these oral conditions. By meticulously selecting participants through stringent inclusion criteria and employing precise sample collection protocols, the study ensured the reliability and accuracy of the data. The findings revealed markedly elevated levels of 8-OHdG in both saliva and serum samples of periodontitis and OSMF patients compared to healthy individuals, underscoring the increased oxidative stress burden in these diseases. The use of enzyme-linked immunosorbent assay (ELISA) for quantifying 8-OHdG provided robust and consistent results, further validated by significant correlations between salivary and serum 8-OHdG levels within each group. Statistical analysis, including ANOVA and post-hoc tests, confirmed the significant differences in oxidative stress markers among the groups, with the highest levels observed in OSMF patients, reflecting the chronic and severe nature of the oxidative damage in this condition. The study's methodology, including the detailed ELISA procedure and rigorous statistical analysis, ensured the credibility of these findings. The implications of these results are profound, suggesting that monitoring 8-OHdG levels in saliva could serve as a non-invasive and practical approach for early detection, disease monitoring, and evaluating therapeutic interventions in periodontitis and OSMF.

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